From DEPARTMENT OF LABORATORY MEDICINE
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NOVEL COMBINATION CHEMOTHERAPY FOR TREATMENT AND CONTROL OF SCHISTOSOMIASIS AMONG SCHOOL CHILDREN

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Novel Combination Chemotherapy for Treatment and Control of Schistosomiasis among School Children

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I dedicate this work to my family, relatives, colleagues and friends!

More importantly to scientists

“If we are serious about universal health coverage, we must intensify our efforts to beat NTDs”

DR. TEDROS ADHANOM GHEBREYESUS
WHO DIRECTOR-GENERAL

ABSTRACT

Despite reported success in reducing severe disease-associated morbidities in endemic countries, praziquantel (PZQ) alone has failed to control and eliminate schistosomiasis, partly due to its poor efficacy against immature/juvenile worms. Artemisinin derivatives are efficacious against juvenile schistosomes. Therefore, combining PZQ with Dihydroartemisinin-piperaquine (DHP) would complement and potentially add to the killing effect of both mature and immature (juvenile) stages of the parasite to improve cure rates and hence hasten control and elimination of the disease (multi-factorial) and delay development of resistance against PZQ. This PhD thesis aimed to assess the efficacy and safety of PZQ and DHP combination therapy for the treatment and control of intestinal schistosomiasis among school children in Busega district, North-western Tanzania.

First, a baseline survey was conducted to assess the prevalence and correlates of intestinal schistosomiasis infection among 830 school-aged children in North-western Tanzania (***paper I***). A high (90.6%) prevalence of intestinal schistosomiasis infection was observed among school children despite several rounds of mass PZQ treatments in the study area. The prevalence of malaria was found to be very low (<2%). Anaemia (24.6%) and undernutrition such as stunting 29.0% and wasting 11.3% continued to be a major burden among this age group. Being male, having loose stool, and being stunted were the factors found to be significantly associated with high egg counts among those infected. Only lower age (≤ 12 years) was significantly associated with intestinal schistosomiasis infection and not malaria, anaemia or undernutrition status.

In ***paper II***, a prospective surveillance study was conducted among 341 infected school children to assess the efficacy and safety of single-dose PZQ for treatment of *Schistosoma mansoni* infection. Follow-up was done at three weeks post-treatment according to WHO guidelines. We observed that PZQ given as a single-dose at 40 mg/kg body weight is still efficacious and safe for the treatment of *Schistosoma mansoni* infection. The overall cure and egg reduction rates were 81.2% and 95.0%, respectively. The incidence of adverse events was 28.5%, with abdominal pain being the most common. Post-treatment abdominal pain and vomiting were significantly associated with pre-treatment infection intensity and anaemia, respectively.

In ***paper III***, a randomized, open-label, non-inferiority clinical trial was conducted to assess the efficacy and safety of PZQ and DHP combination (n = 298) versus PZQ alone (n = 341). This study has found that PZQ and DHP combination therapy is equally safe and more efficacious than PZQ alone for the treatment of intestinal schistosomiasis. At three weeks post-treatment, cure rates were significantly higher in combination therapy (88.3%) than PZQ alone (81.2%) ($p = 0.01$). At eight weeks post-treatment, there was a significant drop in the cure rates in PZQ alone to 63.9% compared to 81.9% in the PZQ and DHP combination therapy ($p < 0.0001$). Egg reduction rates at eight weeks post-treatment were significantly higher in the PZQ and DHP combination therapy (93.6%) than 87.9% in the PZQ alone group ($p = 0.01$). Overall,

30.8% of the study participants experienced mild and transient adverse events, abdominal pain being the most common adverse event. There was no significant difference in the overall incidence of adverse events between treatment groups.

In ***paper IV,*** a two-arm pharmacokinetic study was conducted among 64 treated children to assess drug-drug interaction and its clinical significance between PZQ and DHP. This study has found that the systemic exposure of PZQ and its enantiomers is increased following coadministration with DHP. The geometric means of AUCs and C_{max} of both total PZQ, R-PZQ and S-PZQ were significantly higher among those children treated with PZQ and DHP combination (n = 32) than those who were treated by PZQ alone (n = 32). The 90% confidence interval of the geometric mean ratios of both total PZQ, R-PZQ and S-PZQ were outside the acceptable bioequivalent interval of 0.80, 1.25, indicating higher systemic exposure among those who were treated with PZQ and DHP combination therapy. The increased PZQ bioavailability in the PZQ and DHP combination is an additional mechanism to enhance the cure rates apart from the killing of both mature and immature stages of the parasite.

In ***paper V***, a pharmacogenetics study was conducted among 340 children to assess the effect of genetic variations in CYP450 on plasma drug concentrations, treatment efficacy and adverse events.. We observed a significant association between *CYP2C19* genotype and PZQ concentration, and its metabolic ratio (*trans*-4-OH-PZQ/PZQ). PZQ concentration was significantly higher among children carrying *CYP2C19 (*2, *3)* defective alleles than the wild type (*CYP2C19 *1/*1*) and *CYP2C19 *17* carriers (ultra-rapid metabolizers) ($p = 0.04$). The metabolic ratio was significantly higher among *CYP2C19 *17* carriers than *CYP2C19 (*2, *3)* carriers ($p = 0.01$). There was no significant effect of *CYP3A4*, *CYP2C19* and *CYP2C9* genotypes on schistosomiasis treatment efficacy and adverse events ($p > 0.05$). However, a border line association between *CYP3A5* genotype and adverse events was observed ($p = 0.048$). On multivariate logistic regression analysis, baseline infection intensity was found to be the only significant predictor of adverse events following PZQ treatment.

In **conclusion**, PZQ and DHP combination therapy is equally safe and more efficacious than PZQ alone for treatment of schistosomiasis. The increased systemic PZQ exposure following a co-administration of DHP, yet without affecting overall safety, is an additional mechanism to the observed higher cure rate among children treated with PZQ and DHP combination therapy. These findings call for policy makers and other stakeholders to consider the use of PZQ and DHP combination therapy for the treatment and control of schistosomiasis in endemic countries.

LIST OF SCIENTIFIC PAPERS

The following papers are included in the thesis:

I. **Mnkugwe RH,** Minzi OS, Kinung'hi SM, Kamuhabwa AA, Aklillu E. Prevalence and correlates of intestinal schistosomiasis infection among school-aged children in North-Western Tanzania. ***PloS one***. 2020;15(2):e0228770. Epub 2020/02/06. doi: 10.1371/journal.pone.0228770. PubMed PMID: 32023307.

II. **Mnkugwe RH,** Minzi OS, Kinung'hi SM, Kamuhabwa AA, Aklillu E. Efficacy and Safety of Praziquantel for Treatment of *Schistosoma mansoni* Infection among School Children in Tanzania. ***Pathogens***. 2019;9(1). Epub 2020/01/02. doi: 10.3390/pathogens9010028. PubMed PMID: 31892235.

III. **Mnkugwe RH,** Minzi O, Kinung'hi S, Kamuhabwa A, Aklillu E (2020) Efficacy and safety of praziquantel and dihydroartemisinin piperaquine combination for treatment and control of intestinal schistosomiasis: A randomized, non-inferiority clinical trial. ***PLoS Negl Trop Dis*** 14(9): e0008619.

IV. Minzi, O.M.; **Mnkugwe, R.H**.; Ngaimisi, E.; Kinung'hi, S.; Hansson, A.; Pohanka, A.; Kamuhabwa, A.; Aklillu, E. Effect of Dihydroartemisinin-Piperaquine on the Pharmacokinetics of Praziquantel for Treatment of Schistosoma mansoni Infection. ***Pharmaceuticals*** 2021, 14, 400. https://doi.org/10.3390/ ph14050400

V. **Mnkugwe RH,** Minzi OS, Kinung'hi SM, Kamuhabwa AA, Aklillu E. Effect of pharmacogenetics variations on praziquantel plasma concentration and intestinal schistosomiasis treatment outcomes among infected school-aged children in Tanzania. (***Manuscript*****)**

CONTENTS

LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
ALU	Artemether Lumefantrine
ANOVA	Analysis of variance
AUC	Area under concentration-time profile curve
BAZ	Body mass index (BMI) for Age Z score
BCG	Bacillus Calmette–Guérin
BMI	Body mass index
CDC	Center for Disease Control
C_{max}	Maximum plasma concentration
CRF	Confidential record form
CV	Coefficient of variation
CYP450	Cytochrome P450
DALYs	Disability-adjusted life years
DDI	Drug-drug interaction
DHA	Dihydroartemisinin
DHP	Dihydroartemisinin piperaquine
DNA	Deoxyribonucleic acid
EDTA	Ethylene-Diamine-Tetra-Acetic acid
EPG	Eggs per gram of stool
ERR	Egg reduction rate
GMR	Geometric mean ratio
HAZ	Height for Age Z score
Hb conc	Haemoglobin concentration
HDI	Human development index
HIV	Human immunodeficiency virus
IPT	Intermittent preventive treatment
IQR	Interquartile range
IS	Internal standard
KI	Karolinska Institutet
LC-MSMS	Liquid chromatography-tandem mass spectrometer
LAMP	Loop-mediated isothermal amplification
LBW	Low birth weight
MDA	Mass drug administration

MRCC	Medical Research Coordination Committee
MRDT	Malaria rapid diagnostic test
MUHAS	Muhimbili University of Health and Allied Sciences
NIMR	National Institute for Medical Research
NTDs	Neglected tropical diseases
PACTR	Pan African Clinical Trial Registry
PCR	Polymerase chain reaction
PKNCA	Perform pharmacokinetics non-compartmental analysis
POC CAA	Point of care circulating anodic antigen
POC CCA	Point of care circulating cathodic antigen
PZQ	Praziquantel
R-PZQ	R-praziquantel
SD	Standard deviation
SDGs	Sustainable development goals
SPSS	Statistical Package for Social Sciences
SSA	Sub Saharan Africa
S-PZQ	S-praziquantel
UGTs	UDP-glucuronosyltransferase
UPLC-MSMS	Ultra-performance Liquid chromatography-tandem mass spectrometer
WHO	World Health Organization

1 INTRODUCTION

1.1 THE BURDEN OF NEGLECTED TROPICAL DISEASES

Neglected Tropical Diseases (NTDs) are still a public health challenge, especially among children in Sub-Saharan Africa (SSA) [1-3]. NTDs are a group of poverty-related diseases that prevail in tropical and sub-tropical regions [4]. Currently, the World Health Organization (WHO) recognizes a total of 20 NTDs that have different causative agents, including protozoa, helminths, bacteria, and viruses. NTDs have a major negative socio-economic impact among families and communities, mostly in low and middle-income countries [4]. Thus, the SSA region remains one of the most affected areas [1, 3, 4].

NTDs are associated with disabilities, poor growth, and cognitive performance, prevent children from attending school, thus affecting their future socio-economic life [5]. On the other hand, infected older populations become less involved in economic activities and hence become less productive to their families and community [1, 6]. About 534,000 deaths and more than 57 million Disability-Adjusted Life Years (DALYs) are attributed to NTDs annually [2]. The global burden of DALYs due to NTDs has been reported to be greater than that of malaria (46.5 DALYs) and tuberculosis (34.7 DALYs) [7]. Furthermore, NTDs also affect the human development index's (HDI) key components, such as living standards, quality education and years of schooling, and years of life lived with good health [8].

The healthy and socio-economic consequences of NTDs further fuel the burden of poverty to the families, communities, countries and SSA region. NTDs have also been linked to the delay in the achievement of sustainable development goals (SDGs), e.g. SDG1 (no poverty), SDG3 (good health for all), and SDG4 (quality education) [8, 9]. Earlier studies have shown that treatment and control of NTDs have been associated with a direct impact on the efforts towards achievement of the SDGs [10]. The current global agenda is to eliminate NTDs by the year 2030. To achieve this milestone, further research on treatment optimization and control strategies is needed. Schistosomiasis is among the NTDs targeted for control (heavy infection < 5%) and elimination (heavy infection < 1%) as a public health challenge globally by the year 2025 [11, 12].

1.2 THE GLOBAL BURDEN OF SCHISTOSOMIASIS

Schistosomiasis is a poverty-related NTD caused by a parasite blood fluke or trematode of the genus *Schistosoma* [13]. Several *Schistosoma* species exist; however, only three major species infect humans namely *Schistosoma haematobium, Schistosoma mansoni and Schistosoma japonicum* [13]. Other species are *Schistosoma mekongi* and *Schistosoma intercalatum* that infect other mammals [13]. In many areas of the SSA, *Schistosoma haematobium* and *Schistosoma mansoni* are the predominant species and cause significant morbidity and mortality, making them of public health importance in the region [1, 13].

Schistosomiasis is endemic in 78 countries worldwide [14]. Globally, more than 250 million people are infected, and about 800 million people are at risk of the disease [14, 15]. More than 90% of the reported disease global burden is contributed by cases from SSA, where it causes about 150,000 - 280,000 deaths annually [2, 15]. Schistosomiasis is estimated to cause up to 3.31 million DALYs annually [2]. According to the WHO, by 2017, approximately 99 million

people (81.1 million being school-aged children) received schistosomiasis treatment worldwide [16]. Schistosomiasis is a treatable and preventable disease but, if not treated, may cause renal failure, hydronephrosis, upper gastrointestinal bleeding as a result of portal hypertension, and cancer due to chronic inflammation resulting from migrating parasites eggs in body tissues [1]. These health consequences further increase the burden and cost in the health care system in the affected countries.

Apart from its morbidities, schistosomiasis has been associated with an increased risk of Human Immunodeficiency Virus (HIV) transmission and progression [17-19]. Additionally, studies have also shown that *Schistosoma mansoni* infection increases susceptibility to malaria by changing the balance of T-helper cells 1 and 2 immune responses affecting the immunological protection of malaria [19-22]. In addition to that, the morbidity and severity of parasitic infections, including malaria, increases when in co-infection with schistosomiasis [19, 23]. Schistosomiasis has also been linked to poor pregnancy outcomes due to anemias in pregnancy, which may eventually cause maternal mortality and/or low birth weight (LBW) [24, 25].

Though there is still a debate on the relationship between schistosomiasis and anaemia, some studies have indicated a significant association between schistosomiasis (i.e. *Schistosoma haematobium*) and anaemia among children [26]. Despite the lack of association between schistosomiasis and anaemia in most studies, a high burden of anaemia was reported among those infected with schistosomiasis [27-30]. Additionally, schistosomiasis has also been associated with undernutrition (stunting and wasting) [31, 32], but also the presence of undernutrition (e.g. stunting) is associated with high eggs count among those infected [29]. Studies conducted to assess the efficacy of Bacillus Calmette–Guérin (BCG) vaccination for tuberculosis have reported a reduced protective effect among those infected with *Schistosoma mansoni,* particularly in heavy infections [33]. All these negative outcomes of schistosomiasis can significantly be reduced if the disease is controlled or eliminated. Therefore, there is a need to continuously assess the prevalence of schistosomiasis in endemic countries to inform the responsible authorities such as the national NTDs control and elimination programs for appropriate actions.

1.3 THE EPIDEMIOLOGY OF SCHISTOSOMIASIS IN TANZANIA

The first report of schistosomiasis in Tanzania was reported on intestinal schistosomiasis back in early 1895 [34]. Since then, the distribution and epidemiology of both intestinal schistosomiasis and urogenital schistosomiasis have been reported all over the country, with the level of endemicity and prevalence varying from region to region [35]. The distribution of schistosomiasis is mainly influenced by the availability and distribution of an intermediate host of the parasite (i.e. snails) [35]. *Schistosoma haematobium* was reported to be endemic throughout the country, with the Eastern and South-Eastern coasts of the country being highly affected [36, 37]. On the other hand, *Schistosoma mansoni* was reported to be highly prevalent in the North-western part of the country surrounding Lake Victoria [38, 39]. While in Zanzibar Islands (Unguja and Pemba), *Schistosoma haematobium* is the only endemic species [40]. The prevalence of schistosomiasis has been reported to increase over the years in the country, with an increase in the national population size [35]. In 1979-1980, about 28.3% and 23.2% of the Tanzania mainland population were infected with urogenital and intestinal schistosomiasis,

respectively [41]. In the 1990s, the prevalence of schistosomiasis was reported to increase to about 51.5% [42]. In 2009, about 19 million people of the 43.5 million estimated populations were infected with schistosomiasis [3].

To date, despite ongoing control interventions, schistosomiasis is still endemic throughout the country [43] and highly prevalent around the Lake Victoria Zone, where a prevalence of up to 100% has been reported in some areas (Figure 1) [35]. A study conducted in 2016 to assess the progress of schistosomiasis control globally has reported Tanzania among the countries not yet to achieve schistosomiasis control and elimination (Figure 2) [43]. Additionally, studies conducted within the country in 2016 and 2017 around the Lake Zone have reported an alarmingly high prevalence of intestinal schistosomiasis of > 80% among school children [27, 28]. On the other hand, currently, there is evidence of a high prevalence of schistosomiasis (> 45%) among pre-school children in Tanzania [44]. The pre-school children were not part of mass praziquantel (PZQ) treatment campaigns in the country and, therefore, served as a reservoir of infection for many years.

Though schistosomiasis has not been adequately studied among pregnant women, a high prevalence of more than 60% has been reported among pregnant women in Tanzania [25]. This overall burden of schistosomiasis in different age groups makes Tanzania the second most affected country by the disease next to Nigeria among the African countries [45]. Therefore, there is a need to continue monitoring the prevalence of schistosomiasis in the country across all age groups. Assessment of disease prevalence is the only way to inform the national NTDs control programs on the progress of the ongoing interventions [46].

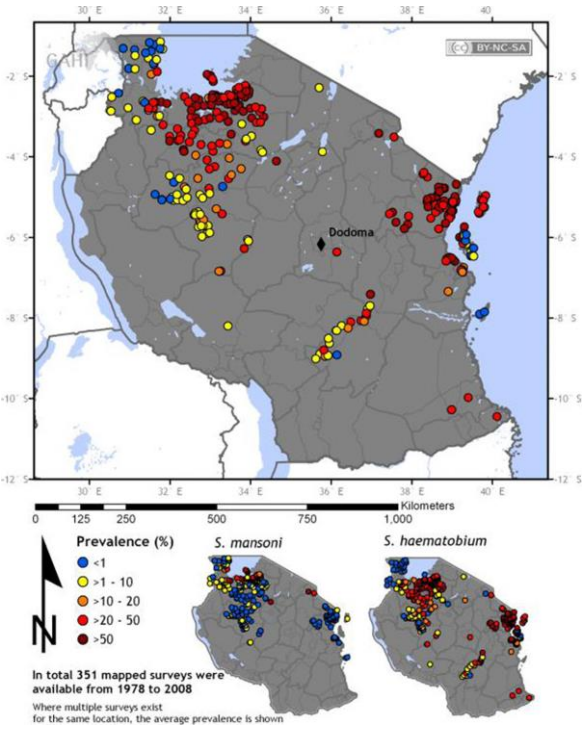

Figure 1*: Map of Tanzania showing the distribution of both Schistosoma mansoni and Schistosoma haematobium. Source: Mazigo et al., 2012 [35].*

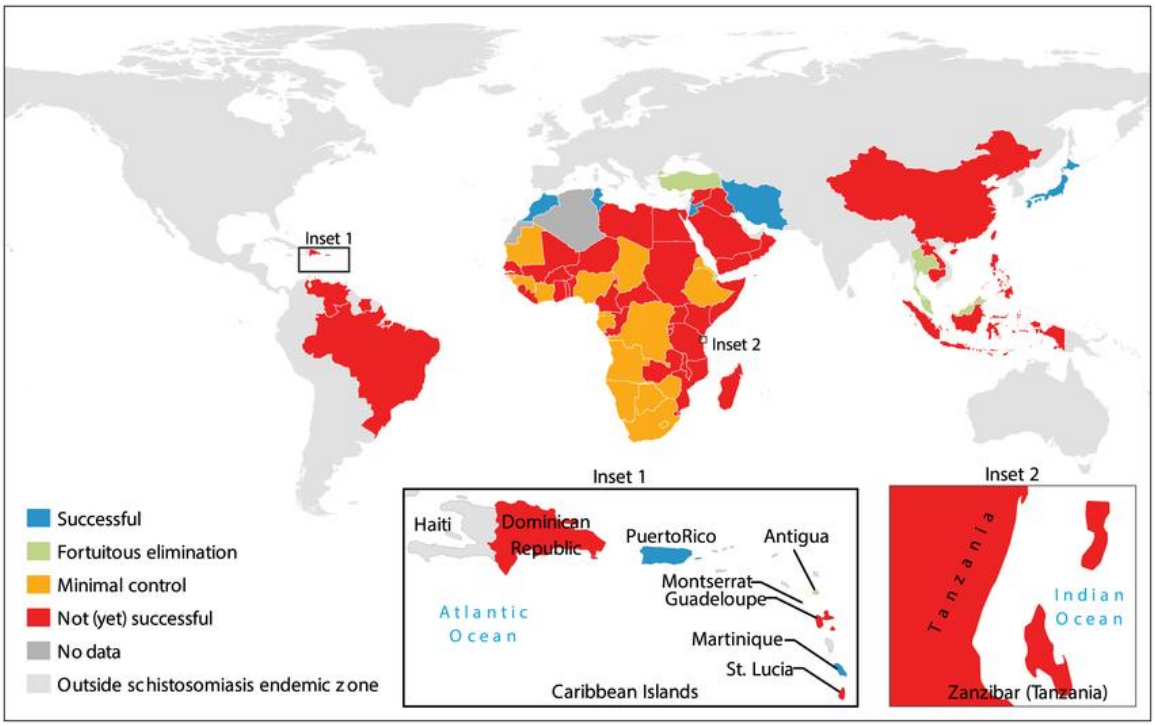

***Figure 2**: A global map showing the outcome of schistosomiasis control and elimination as of 2016. Tanzania is among the SSA countries not yet succeeded in achieving schistosomiasis control. Source: Sokolow SH et al., 2016 [43].*

1.4 LIFE CYCLE AND TRANSMISSION OF SCHISTOSOMIASIS

Schistosomes have two different hosts and two developmental stages in their life cycle (mature and immature stages) [47]. The human being is the definitive host and acquires the infection through contaminated freshwater bodies (lakes, dams, ponds, and rivers) with an infective larvae stage of the parasite called cercariae [47]. The cercariae penetrate human skin and develop into immature or juvenile worms called schistosomula [47]. This stage of the parasite is less affected by PZQ, the drug currently used for the treatment of schistosomiasis [48].

The immature schistosomula are then carried in the blood to the portal vein of the liver, where they develop into mature adult worms and this process takes about 4 to 6 weeks [1, 47]. After that, a pair of matured female and male adults migrates against venous blood from the liver (portal vein) to the perivesical venous plexus of the bladder (*Schistosoma haematobium*) and venous plexus of mesenteries of the bowel and portal veins (*Schistosoma mansoni*) [47]. Within these venules, female parasites start to lay up to several hundred eggs per day for several years. Adult schistosome parasites can survive for 3 to 10 years, while eggs can survive for 1 to 2 weeks [47].

The eggs penetrate the tissue causing intensive inflammation and organ damage, while other eggs reach the lumen of the bladder (*Schistosoma haematobium*) or colon (*Schistosoma mansoni*) excreted in either urine or stool, respectively. The excreted eggs hatch in freshwater to release miracidia which penetrate the freshwater snails (intermediate host), multiplying by asexual reproduction to form cercariae, the infective stage of the parasite. This process takes about 4 to 6 weeks [47]. Then the infective cercariae are released into water to search for a definitive human host to complete the cycle (Figure 3). Intermediate snail hosts of the *Bulinus*

species are responsible for transmission of *Schistosoma haematobium* while *Biomphalaria* species are responsible for transmission of *Schistosoma mansoni* [49].

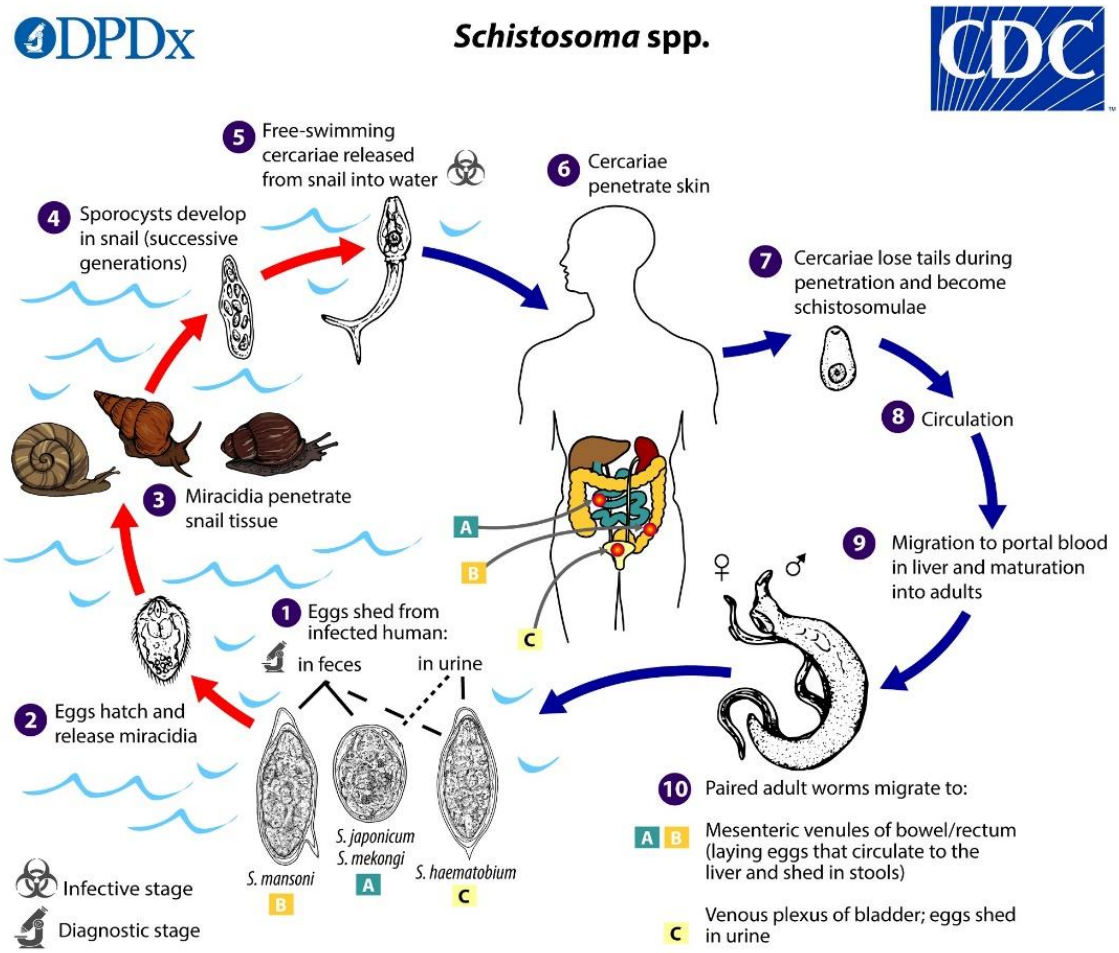

***Figure 3**: Life cycle of Schistosoma. Source: Centers for Disease Control and Prevention (CDC): Source: [http://www.cdc.gov/dpdx/schistosomiasis/.](http://www.cdc.gov/dpdx/schistosomiasis/)*

1.5 CLINICAL MANIFESTATIONS OF SCHISTOSOMIASIS

Most infected people in endemic countries are asymptomatic. In general, schistosomiasis causes disabilities more than it kills. Most morbidities associated with schistosomiasis result from the body's immune reaction to the migrating parasite eggs [50]. Schistosomiasis has three main clinical phases, whereby the first phase is characterized by dermatitis and is caused by the penetrating cercariae [51]. The second phase is marked by fever (Katayama fever) and other constitutional complaints, e.g., headache, fatigue, cough due to migrating schistosomula and eggs [52]. The last phase results in chronic inflammation and tissue damage which is caused by parasite eggs trapped into host tissue [53]. Table 1 summarizes the clinical phases and symptoms of the disease.

Table 1. Clinical phases and symptoms of schistosomiasis

Clinical phase	Symptoms
Immediate	Acute, pruritic, maculopapular eruption at site of cercarial skin penetration within 1 day following exposure
Acute	Systematic hypersensitivity reaction against migrating schistosomula, fever, fatigue, myalgia, malaise, nonproductive cough, eosinophilia, patchy infiltrates, weight loss, dyspnea, diarrhea, diffuse abdominal pain, toxemia, hepatosplenomegaly, widespread rash
Chronic	Affects gastrointestinal and urogenital tracts, leading to hepatosplenic and pelvic organ diseases, portal and pulmonary hypertension, abdominal ascites, upper gastrointestinal varices and hemorrhage, female genital schistosomiasis, infertility, increased risk of HIV-1 transmission, and squamous cell carcinoma of the bladder

Source 'Vale N *et al*., 2017 [54].

Classic symptoms of intestinal schistosomiasis include abdominal pain, diarrhoea or bloody diarrhoea [53]. Enlargement of the liver (hepatomegaly) occurs mostly in the advanced stage of the disease [50]. Hepatomegaly is frequently accompanied by fluid in the peritoneal cavity and raised blood pressure in the portal veins (portal hypertension), which may further cause upper gastrointestinal bleeding as a complication [50]. Hepatomegaly is also associated with enlargement of the spleen (splenomegaly) in advanced cases [50]. If not treated, intestinal schistosomiasis associated with health consequences, including anaemia, growth retardation (stunting and wasting), decreased physical fitness, and poor cognitive development. These health consequences can negatively impact children's ability to learn [1]. Therefore, infected children will not be able to achieve life potentials, particularly in endemic countries with extreme poverty.

For urogenital schistosomiasis, the classic clinical sign is blood in the urine (haematuria) [50]. Kidney damage and urinary bladder fibrosis may occur in the advanced stages of the disease [53]. The latter may progress to urinary bladder cancer in some patients [50]. Urogenital schistosomiasis can also affect reproductive organs and may cause infertility in both sexes [55].

Therefore, it is important to continuously assess schistosomiasis-associated co-morbidities, including anaemia and nutritional deficiencies in future studies that will be conducted to assess the prevalence of schistosomiasis. However, all health consequences mentioned above associated with schistosomiasis can be prevented by administering preventive chemotherapy to people at risk in endemic areas such as school-aged children and fishermen.

1.6 DIAGNOSIS OF SCHISTOSOMIASIS

The current strategy for schistosomiasis control and elimination is preventive chemotherapy by mass drug administration (MDA) using PZQ. The assumption is that in such settings, all or most people are infected with the disease. However, in a situations where the diagnosis is important, e.g. individual case management in clinics, in epidemiological studies or in studies conducted to assess drug efficacy, diagnosis of schistosomiasis can be made by microscopic

examination of schistosome eggs in stool or urine samples using the Kato-Katz method (intestinal schistosomiasis) or urine filtration method (urinary schistosomiasis). DNA detection, antigen tests such as the point of care circulating cathodic or anodic antigen (POC CCA/CAA) and molecular diagnostics such as the loop-mediated isothermal amplification (LAMP) or polymerase chain reaction (PCR) can also be used [53].

The non-microscopy-based tests are more sensitive in diagnosing schistosomiasis [56, 57], the WHO still recommends microscopic examination as a gold standard method for diagnosis of schistosomiasis, especially in resource-limited settings [12, 58]. Multiple stool samples collected on two or three consecutive days have been reported to increase the sensitivity of the Kato Katz method, especially in clinical trials; however, in routine disease surveillance, this approach may be costly [53]. In endemic countries with limited resources, the diagnostic test's choice greatly depends on its simplicity, accuracy, less laborious and, most importantly, cost [12, 59]. At the disease elimination stage or in very low infection intensity, it is very important to have more sensitive diagnostic tools for disease surveillance [56, 60]. Although the more sensitive tests are expensive but at the elimination stage, the cost has been reported to be outweighed by the long-term benefits [11, 58]. Therefore, affected countries working towards controlling and eliminating schistosomiasis must be prepared for this cost in advance.

1.7 SCHISTOSOMIASIS TREATMENT, PREVENTION AND CONTROL STRATEGIES

The WHO promotes five main public health interventions against schistosomiasis [12, 61]. These interventions include preventive chemotherapy, snail control and access to safe and clean drinking water, basic sanitation and hygiene, and health education. However, due to financial difficulties in most endemic countries, including Tanzania, preventive chemotherapy targeting school-aged children is the main strategy for treating, preventing, and controlling schistosomiasis [62]. This strategy has been long implemented despite the reported evidence that snail control gives the best outcomes in terms of long-term schistosomiasis prevalence reduction and possible elimination [14, 43]. Periodic mass PZQ treatment given to infected and at-risk populations is the WHO recommendation for treatment, prevention and control of schistosomiasis. In Tanzania, mass PZQ treatment targeting school-aged children was started in the year 2004/2005 [63].

1.7.1 Preventive chemotherapy and its goals in schistosomiasis treatment and control

Preventive chemotherapy is a key strategy recommended by the WHO to treat, prevent and control helminths infections, including schistosomiasis [62]. It is the most preferred strategy due to its logistic feasibility during implementation and cost-effectiveness. Preventive chemotherapy involves large-scale mass antihelminthic drug administration to communities at risk of the disease in endemic countries. In schistosomiasis, preventive chemotherapy with the large-scale use of PZQ is the WHO-recommended strategy. Since 1984, PZQ has been reported as a safe, well-tolerated, and efficacious drug against all *Schistosoma* species [61]. In most endemic countries, including Tanzania, preventive chemotherapy is mainly focused on school-aged children. The frequency of PZQ preventive chemotherapy depends on the prevalence of the disease [64]. Table 2 presents the frequency of PZQ MDA as recommended by the WHO.

Table 2. The WHO recommended PZQ preventive chemotherapy based on thresholds of the prevalence of schistosomiasis infection

Category	Baseline prevalence among school-age children	Action to be taken	
High-risk community	50% by parasitological methods (intestinal and urogenital schistosomiasis) or 30% by questionnaire for history of haematuria	Treat all school-age children (enrolled and not enrolled) once a year	Also treat adults considered to be a risk (from special groups to entire communities living in endemic areas)
Moderate-risk community	10% but < 50% by parasitological methods (intestinal and urogenital schistosomiasis) or <30% by questionnaire for history of haematuria	Treat all school-age children (enrolled and not enrolled) once every 2 years	Also treat adults considered to be at risk (special groups only)
Low-risk community	<10% by parasitological methods (intestinal and urogenital schistosomiasis)	Treat all school-age children (enrolled and not enrolled) twice during their primary schooling age (e.g. once on entry and once on exit)	Praziquantel should be available in dispensaries and clinics for treatment of suspected cases

Source: Schistosomiasis: progress report 2001 - 2011, strategic plan 2012 – 2020 [12]

The initial aim of preventive chemotherapy was morbidity control to reduce severe morbidities associated with the disease [64]. After several years of mass PZQ administration and decline in severe morbidity associated with the disease [65], in 2012, WHO recommended its member states to think beyond reducing severe morbidities to elimination [11, 66]. Despite this effort from the WHO, PZQ alone treatment seems not sufficient enough to control and eliminate schistosomiasis [35]. Several reasons have been reported for the continued high schistosomiasis transmission and prevalence, including lack of vaccine [67], lack of safe and clean water [68], poor sanitation and hygiene [69], and treatment coverage that excluded other key populations such as pre-school children.

More importantly, with preventive chemotherapy itself, the inability of PZQ to act against immature or juvenile worms (schistosomula) has been reported as one of the reasons responsible for continued high schistosomiasis transmission and prevalence [48]. In addition, a threat of PZQ resistance has been reported in field studies in SSA [70]. Therefore, there is a need to continue monitoring the efficacy and safety of PZQ for early detection of resistance but also the need for treatment optimization research to find a better alternative treatment to PZQ. Due to the alarming threat of PZQ resistance, the new alternative treatment should not only improve schistosomiasis treatment efficacy but also reduce or delay the development of resistance against PZQ.

1.7.2 Efficacy and safety of praziquantel for treatment of schistosomiasis

Following several years of PZQ use in schistosomiasis endemic countries, low cure rates, and drug tolerance/resistance have been reported in field studies conducted in SSA [70]. Cure rates following single-dose PZQ treatment at a dose of 40 mg/kg body weight have been reported to range between 42 and 79% for *Schistosoma mansoni* and between 37 and 93% for *Schistosoma haematobium* in school-aged children [71]. In some studies, a cure rate as low as 18% has been reported in Senegal, raising the concern about the current efficacy of PZQ, particularly against *Schistosoma mansoni* infection [70]. These reports indicate the need for regular monitoring of the efficacy of PZQ for the early detection of drug resistance. So far, there is no confirmed or documented report of PZQ resistance [72]. The fact that there is no alternative drug for the treatment and control of this poverty-related disease makes close monitoring of its performance vital.

In Tanzania, despite 100% geographical coverage, repeated cycles of targeted MDA focusing on school-aged children, and the reported national treatment coverage of > 90% [73], the prevalence of schistosomiasis remains high, especially with *Schistosoma mansoni* infection around the Lake Victoria Zone [27-29]. PZQ has been reported to have high efficacy against *Schistosoma haematobium* [74] than *Schistosoma mansoni* infection [75]. Therefore, monitoring PZQ efficacy against *Schistosoma mansoni* may need to be prioritized in endemic areas where both *Schistosoma* species are endemic, like in Tanzania [35]. Also, the WHO recommends assessing drug efficacy if the prevalence and intensity of infection remain high despite preventive chemotherapy [46], and this has been the case for *Schistosoma mansoni* infection around the Lake Victoria Zone in Tanzania [27-29, 35]. Data regarding the current efficacy of PZQ MDA against *Schistosoma mansoni* infection remains scarce in the country.

PZQ is a chiral compound and racemic mixture of R-praziquantel (R-PZQ) and S-praziquantel (S-PZQ) enantiomers. Recent studies have indicated that R-PZQ is pharmacologically active and contributing to the antischistosomal activity of PZQ [76-78]. S-PZQ has been reported to increase the size of the PZQ tablet and responsible for the bitter taste [79]. Studies to investigate the use of monoenantiomeric R-PZQ formulation for the treatment of schistosomiasis especially in children are in pipeline.

Like any other drug, PZQ use has been associated with adverse events in some populations [80]. The adverse events related to PZQ have been broadly categorized as (i) dermatological (e.g. itching, skin rashes, urticaria), (ii) gastrointestinal (e.g. abdominal pain, nausea, vomiting, diarrhoea), (iii) neurological (e.g. muscle pain, headache, dizziness, drowsiness, fainting), and lastly allergic symptoms [80]. Most of these PZQ associated adverse events were reported to be mild and resolve within 24 hours of drug intake [80, 81]. PZQ related adverse events have also been reported to be associated with pre-treatment infection intensities, age, and other medical conditions such as poor nutritional status and anaemia [80, 81]. Heavily infected patients were reported to encounter significantly more adverse events than those with light to moderate infection [80, 81]. Since the treatment-associated adverse events are associated with pre-treatment infection intensity, there is a need for regular monitoring of the safety of PZQ in MDA.

The WHO recommends ensuring safety in large-scale MDA programs involving the use of antihelminthic drugs [82]. Currently, there are large-scale donations of antihelminthic drugs,

including PZQ, from pharmaceutical companies and global health organizations under the influence of the WHO for the treatment and control of NTDs worldwide. Although this scale-up in PZQ donations is necessary, it also calls for regular monitoring of the drug efficacy and safety [83].

1.8 ALTERNATIVES TREATMENT STRATEGIES TO SINGLE-DOSE PRAZIQUANTEL

To date, there is no approved vaccine in the market for schistosomiasis prevention, although potential candidates are on clinical trials [48, 67]. Likewise, there are no new antischistosomal drugs approved for the treatment and control of schistosomiasis. With the reported threat of PZQ resistance, there is a pressing need for a new treatment alternative. Optimization of available drugs in the market remains the only option in the era of schistosomiasis control and elimination worldwide [84]. Several treatment strategies have been tested in an attempt to optimize schistosomiasis treatment outcomes, including the use of higher PZQ doses [85, 86], repeated PZQ doses [75, 87-89], and combination chemotherapy [90]. However, each strategy has shown its weaknesses or limitations, as detailed below. Hence the need for exploring other treatment options [56].

1.8.1 Higher praziquantel doses

Higher PZQ doses (> 40mg/kg body weight) have been tested and demonstrated contradicting findings regarding egg clearance/efficacy. They have shown improved cure rates in some studies [86] while no beneficial effects in other studies [85]. Despite success in some studies, higher PZQ doses have been associated with significantly more treatment-associated adverse events than the standard dose of 40mg/kg body weight [86]. Therefore, this strategy was not adopted for use due to safety concerns. Indeed, with the current wide implementation of targeted large-scale preventive chemotherapy for the control and elimination of NTDs, the safety of administered drugs to the affected populations is the WHO recommendation [82] and critically important for treatment coverage and MDA compliance [56].

1.8.2 Repeated praziquantel treatment strategy

The utility of repeated PZQ doses in the space of 3-6 weeks has been investigated in endemic countries to kill the left behind parasites that were immature during the first round or dose of PZQ MDA [88]. This way of administering PZQ has shown success in improving cure rates, particularly in high disease transmission areas [87, 88]. Although there is still ongoing debate regarding the usefulness of repeated PZQ doses versus cost and the risk of accelerating PZQ resistance, the use of this strategy in high schistosomiasis transmission areas where a single annual PZQ dose is obviously not adequate cannot be underestimated.

However, repeated PZQ doses strategy will definitely increase PZQ use, and previous studies have reported that increased PZQ MDA rounds have been linked to low cure rates and may also increase the risk of PZQ resistance [91]. Furthermore, repeated PZQ doses strategy has potential for poor implementation, especially that schistosomiasis affects developing countries with limited resources. A survey of experts towards NTDs eliminations has indicated a lack of resources as a major challenge in schistosomiasis control and elimination in endemic countries

[92]. Therefore, repeated PZQ doses strategy, despite its reported success in improving the cure rates, may not be cost-effective in endemic countries with limited resources.

1.8.3 Combination chemotherapy for schistosomiasis

Like in other infectious diseases such as HIV, tuberculosis and malaria, combination therapy remains the only hope and feasible strategy to optimize schistosomiasis treatment outcomes [90]. In fact, like in other infectious diseases, the combination therapy strategy will not only improve treatment outcomes but also will reduce or delay the development of resistance against PZQ provided that drugs have independent mechanisms of action and/or act on a different developmental stage of the parasite [90, 93-95].

1.8.3.1 PZQ and Oxamniquine Combination therapy

Combination chemotherapy of PZQ and oxamniquine has been investigated but the findings have not shown convincing evidence of superior benefit to PZQ alone [90, 96]. Not only oxamniquine is ineffective against the immature stage of the parasite like PZQ but also it is only effective against *Schistosoma mansoni* [90]. Therefore, the combination of PZQ and oxamniquine was not recommended for use in the treatment and control of schistosomiasis worldwide due to limited broad antihelminthic activity against different parasite stages and species.

1.8.3.2 Combination therapy of PZQ and artemisinin derivatives

PZQ is not effective against immature or juvenile worms [46, 48]. Mass PZQ administration leaves behind the immature parasites, which after 4 to 6 weeks, they grow to the matured stage and start laying eggs [1]. Hence the prevalence and intensity of infection may go back to the pre-treatment levels. As indicated earlier, this shortcoming of PZQ has partly contributed to the failure to control and eliminate schistosomiasis in endemic countries. A broader antihelminthic approach supplementing PZQ with a new antischistosomal drug targeting different parasite development stages and/or having a different mechanism of action would not only increase the efficacy but also reduce the risk for PZQ resistance [93].

Artemisinin derivatives (e.g. Artemether, Artesunate, and Dihydroartemisinin) have been reported to have high efficacy against immature or juvenile worms apart from their antimalarial activity [48]. Artemisinin combined with PZQ has been tested to treat schistosomiasis and has shown success in improving the cure rates [97-99]. PZQ and artemether or artesunate combination have performed better in schistosomiasis treatment than PZQ alone, and the combination has been reported suitable for the treatment of patients with repeated exposure to infected water [98, 99]. Dihydroartemisinin, a derivative of artemisinin has shown high activity against juvenile worms (schistosomula) of *Schistosoma mansoni* [100]. However, a concern is raised on artemisinin use as monotherapy combined with PZQ in settings where malaria is also endemic, like SSA, due to the risk of developing artemisinin resistance in malaria parasites [101]. Artemisinin derivatives remain key drugs for the treatment of malaria worldwide [102].

Therefore, in malaria-endemic areas, the use of artemisinin-based combination therapy (ACTs) combined with PZQ is a better option as the risk of artemisinin resistance is greatly reduced [72]. In such combination, artemisinin is protected by a long-acting partner drug, e.g., piperaquine or Lumefantrine. In fact, ACTs alone have shown high efficacy (100% cure rate)

against schistosomiasis when given to treat malaria in patients co-infected with schistosomiasis, with a small sample size being the limitation in those studies [103-105]. Currently, there is no data on the treatment efficacy and safety of the combination of PZQ and ACTs, such as DHP for the treatment and control of schistosomiasis. In this PhD thesis, the efficacy and safety of PZQ and DHP combination therapy versus PZQ alone has been investigated for the treatment and control of intestinal schistosomiasis. DHP is a first-line antimalarial drug used for the treatment of uncomplicated malaria [102]. DHP was chosen over other ACTs (e.g. Artemether Lumefantrine) because of its easy schedule of administration (once per day for three days) [102] and has not extensively used for malaria treatment in most parts of SSA, including Tanzania [106] hence less drug pressure. DHP also has long post-prophylaxis protection against malaria, which will offer protection to children who remain to be a vulnerable population against malaria in SSA [107].

1.9 DRUG-DRUG INTERACTION BETWEEN PRAZIQUANTEL AND DIHYDROARTEMISININ-PIPERAQUINE

In general, many factors may influence treatment outcomes, including the drug's inability to eliminate a parasite in one of its developmental stages of the life cycle, sub-optimal drug concentrations, use of sub-standard drugs, drug-drug interaction, individual genetics, and drug resistance [46, 93]. PZQ is sourced from the WHO pre-qualified drug companies; therefore, it is unlikely that the available PZQ in the market is sub-standard, although this cannot be guaranteed by 100%. On the other hand, much is still unknown about the pharmacokinetic of PZQ (i.e., plasma drug levels) in schistosomiasis infected children when used as a single dose [108]. With the introduction of combination therapy for schistosomiasis treatment, like in any drug combination therapy, there is a risk of drug-drug interactions if the combined drugs share the same metabolic pathway [109]. Drug-drug interactions may increase or decrease PZQ plasma concentration and further compromise schistosomiasis cure rates and adverse events profile [78].

Dihydroartemisinin (DHA), an artemisinin derivative, is mainly metabolized via glucuronidation catalyzed by UDP-glucuronosyltransferases (UGTs), in particular UGT1A9 and UGT2B7 [110]. While both PZQ and piperaquine (PPQ) (aminoquinoline derivative) are metabolized by cytochrome P450 enzymes (CYP450), e.g. CYP3A4/5, and this poses a risk for drug-drug interactions [111, 112]. Chloroquine, which is also an aminoquinoline derivative similar to PPQ [113], has been found to reduce the bioavailability and maximum concentration (C_{max}) of PZQ in humans [114].

Therefore, it is important to assess pharmacokinetic interactions of clinical significance when PZQ and DHP are used in combination chemotherapy. To the best of our knowledge, no study has assessed drug-drug interaction between PZQ and DHP. A review done by Utzinger J *et al* to assess combination chemotherapy of schistosomiasis recommended measurement of drug levels of the drugs used in combination therapy to treat schistosomiasis so that a link can be made with treatment outcomes both therapeutic efficacy and safety [90]. This PhD thesis investigated the effect of DHP on the pharmacokinetics of PZQ among infected school children to cover the existing gap.

1.10 EFFECT OF PHARMACOGENETICS VARIATIONS ON PRAZIQUANTEL PLASMA CONCENTRATION AND SCHISTOSOMIASIS TREATMENT OUTCOMES

PZQ is primarily metabolized by CYP450 enzymes, including CYP3A4, CY3A5, CY2C19 and CYP2C9 [111]. Most of these metabolizing enzymes are polymorphic, displaying inter-individual variability in enzyme optimal function [115]. The change in enzyme activity due to genetic polymorphism in some individuals may affect plasma drug concentration, treatment efficacy and the incidence of treatment-associated adverse events [115]. CY3A4 has been described as a major metabolizing enzyme for most drugs used to treat tropical infections [115]. In malaria treatment, the *CYP3A4* genotype was associated with treatment outcomes among the Tanzanian population treated with Artemether Lumefantrine [116]. On the other hand, *CYP3A5* has been reported to be expressed mostly in the black population and is considered the most pharmacologically active CYP450 in the African population [117]. Studies have reported that most inter-population differences in *CYP3A5* genotype are due to *CYP3A5*3* [115]. The lowest frequency of mutation in *CYP3A5*3* has been reported in the Sub-Saharan population [115].

The sub-family CYP2C has been reported to metabolize about 20% of all CYP450 substrates. CYP2C9 and CYP2C19 are among the pharmacologically active CYP enzymes in the CYP2C sub-family and are also polymorphic. For example, for CYP2C19, about 3.6% of the Tanzanian population have been phenotyped as poor metabolizers in previous studies [118].

Studies conducted in other infectious diseases such as HIV, Tuberculosis, and malaria have shown the influence of genetic variations on plasma drug concentrations and treatment outcomes, both efficacy and safety [116, 119-122]. However, data on the effect of pharmacogenetics variations on PZQ plasma concentration and schistosomiasis treatment outcomes are lacking [78, 123]. To the best of our knowledge, no pharmacogenetics study has been conducted to assess the relevance of pharmacogenetics variations on PZQ plasma concentration and schistosomiasis treatment outcomes despite the reported variability in plasma PZQ concentration, cure rates, and adverse events profile between populations in previously reported studies [80, 87].

Although the current approach of schistosomiasis treatment and control using MDA is a challenge for the individualization of treatment (genotype-based), data on the effect of pharmacogenetics variations on PZQ plasma concentration and schistosomiasis treatment outcomes are necessary for immediate future use. Pharmacogenetics data utilization efforts have also been recently intensified in Africa [124]. This PhD thesis investigated the role of pharmacogenetics variations on PZQ plasma concentration and schistosomiasis treatment outcomes, both efficacy and safety to cover the existing gap.

2 AIM OF THE RESEARCH

The main aim of the PhD thesis was to optimize schistosomiasis treatment outcomes among infected school children. This was done through a randomized, open-label, non-inferiority clinical trial to assess the efficacy and safety of PZQ and DHP combination therapy against PZQ alone for treatment and control of intestinal schistosomiasis.

2.1 SPECIFIC OBJECTIVES

1. To investigate the prevalence of intestinal schistosomiasis and its association with malaria, anaemia and nutritional status among school children in North-western Tanzania (*paper 1*)

2. To assess the efficacy and safety of single-dose PZQ for treatment of *Schistosoma mansoni* infection among school children in North-western Tanzania (*paper 2*)

3. To assess the efficacy and safety of PZQ and DHP combination therapy versus PZQ alone for treatment of *Schistosoma mansoni* infection among school children (*paper 3*)

4. To assess pharmacokinetic drug-drug interaction and its clinical relevance between PZQ and DHP following coadministration for treatment of intestinal schistosomiasis (*paper 4*)

5. To investigate the effect of pharmacogenetics variations on PZQ plasma concentration and intestinal schistosomiasis treatment outcomes (*paper 5*)

3 MATERIALS AND METHODS

3.1 STUDY SETTING

The field work for the PhD research project was conducted in Nyamikoma village, Busega district, Simiyu region in North-Western Tanzania in collaboration with the National Institute for Medical Research (NIMR) Mwanza research centre (Figure 4). The North-western region of Tanzania around the Lake Victoria basin is one of the highly affected areas in the country, particularly with *Schistosoma mansoni* infection (Figure 1) [35]. Nyamikoma village, a rural area, located at latitude 2° 19' 59" S and longitude 33° 40' 59" E along the shores of Lake Victoria, was purposely selected for this study due to high endemicity of intestinal schistosomiasis and accessibility to NIMR Mwanza research centre. The project involved collecting blood and plasma samples that needed to be stored at -80°C on daily basis and therefore, accessibility of the study site was vital for the success of sample transportation and storage.

According to the Tanzania 2012 population and housing census, the Busega district had about 203,597 people [125]. The district has two rainfall patterns, light rain from October to December and heavy rain from March to May of every year. The mean temperature is between 18°C to 20°C during the rainy season and up to around 32°C in the dry season. The dominant tribe is Sukuma though other tribes such as Kurya and Jita are found. Fishing and farming of both crops and animals are the main economic activities around the district. Lake Victoria is the main source of water for all human needs in the study area.

The laboratory work (pharmacokinetics and pharmacogenetics analysis) was done at the Department of Laboratory Medicine, Karolinska University Hospital Huddinge (Stockholm, Sweden).

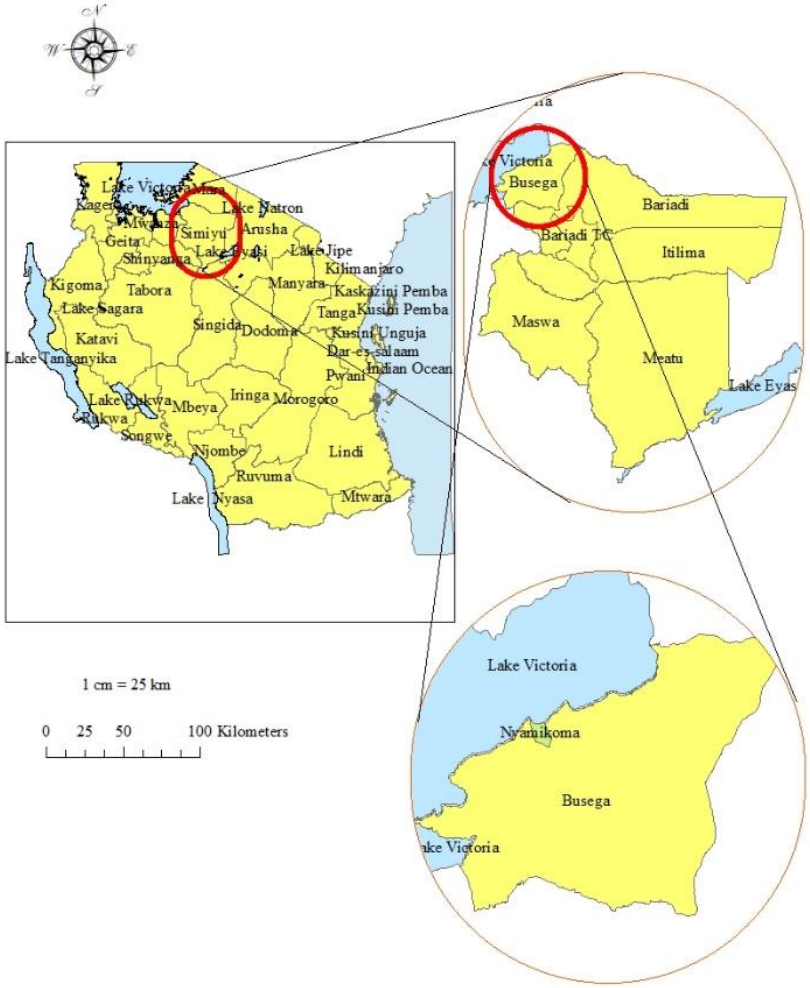

Figure 4*: Map of Tanzania showing Simiyu Region and the study site (Nyamikoma village) located along the shores of Lake Victoria in Busega district*

3.2 STUDY DESIGN AND POPULATION

To achieve the overall goal of the PhD project, different study designs and populations were used in the sub-studies, as presented in Figure 5. In sub-study 1, a cross-sectional study was conducted among 830 eligible school-aged children to assess the prevalence and correlates of intestinal schistosomiasis (*paper I*). In sub-study 2, an efficacy and safety surveillance study was conducted among children who tested positive for *Schistosoma mansoni* infection and were treated with single-dose PZQ (n = 341) (*paper II*). Sub-study 3 was a randomized, open-label, non-inferiority clinical trial among infected children who were treated with either PZQ alone (n = 341) or PZQ plus DHP combination therapy (n = 298) (*paper III*). In sub-study 4, a two-arm pharmacokinetic study was conducted on a randomly selected sample of 64 children treated with PZQ + DHP combination (n = 32) and PZQ alone (n = 32) to assess drug-drug interaction between PZQ and DHP and its clinical significance (*paper IV*). Sub-study 5 was a pharmacogenetics-pharmacokinetics-pharmacodynamics study conducted among children involved in sub-study 2 (*paper II*) to assess the impact of pharmacogenetics variations on PZQ plasma concentrations and intestinal schistosomiasis treatment outcomes (*paper V*).

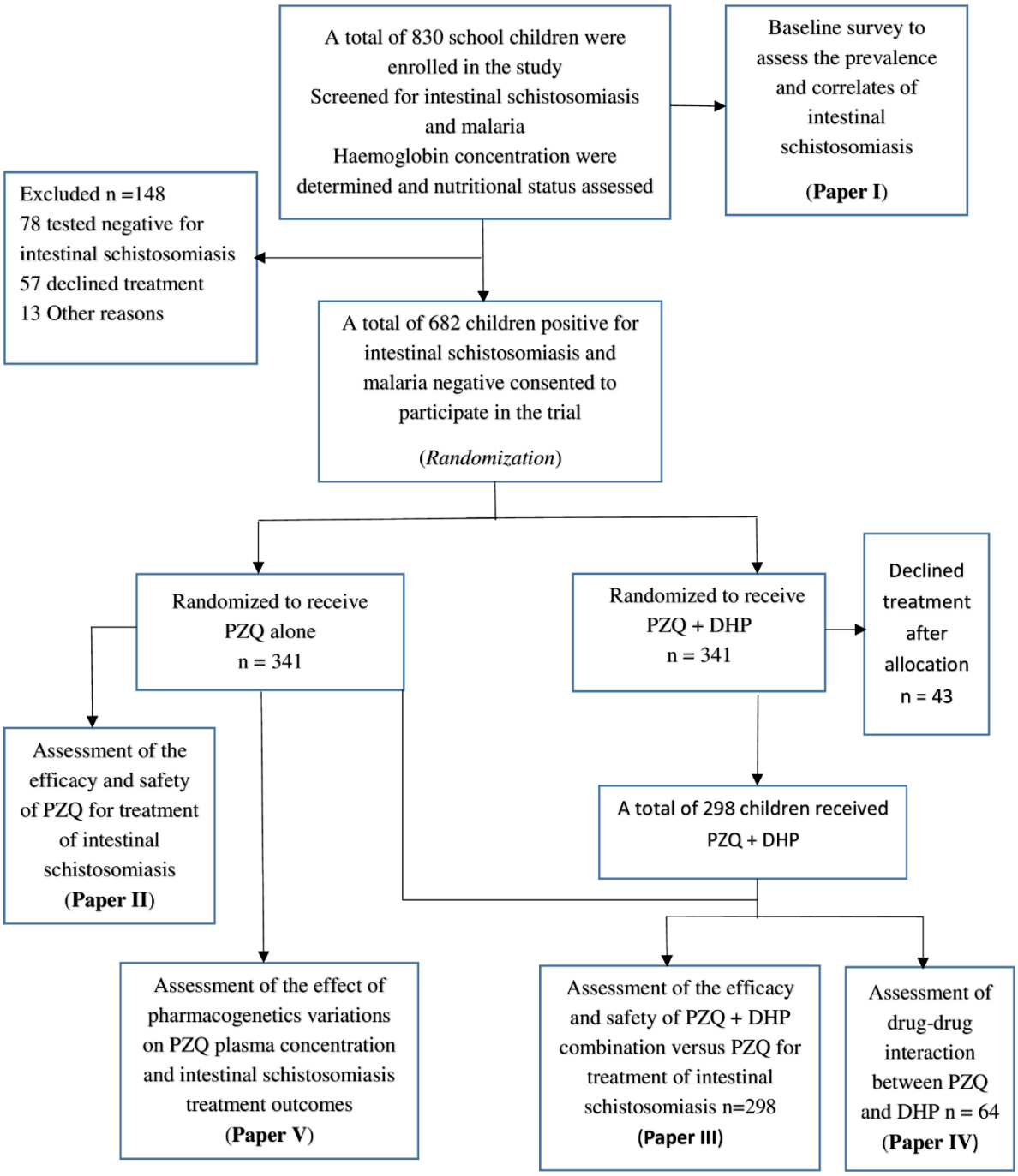

***Figure 5**: Study flow chart showing study population enrolled in each sub-studies in the project*

3.3 DATA COLLECTION METHODS

3.3.1 Collection of stool samples, processing and examination of *Schistosoma mansoni* infection

Two fresh stool samples were collected on consecutive days from each enrolled participant, both before treatment (*paper I*) and after treatment (*paper II, III and V*). Following treatment, stool samples were collected at three weeks (*paper II & V*) and eight weeks (*paper III*) after treatment to assess drug efficacy [46, 90]. Two duplicate thick slides were prepared from each stool sample using the Kato Katz method [126]. Microscopic examination of the prepared slides was done 24 hours after preparation to allow adequate staining of the schistosome' eggs (with methylene blue) [127]. The intensity of infection or egg per gram of stool (epg) for each study participant was calculated by multiplying the average eggs count from all four slides with a constant factor of 24. Baseline infection intensity was classified according to the WHO guidelines [12] as presented in Table 3. Ten percent of the slides, both positive and negative for *Schistosoma mansoni* infection were re-examined by an external technician for quality assurance [128, 129].

Table 3. The WHO classification of the intensity of schistosomiasis infection

Organism	Light-intensity infections	Moderate-intensity infections	Heavy-intensity infections
Schistosoma mansoni	1 – 99 epg	100 – 399 epg	≥ 400 epg
Schistosoma haematobium	< 50 eggs/10 ml		≥50 eggs/10 ml

Source: Schistosomiasis: progress report 2001 - 2011, strategic plan 2012 – 2020 [12].

3.3.1.1 Assessment of drug(s) efficacy following PZQ treatment

In *paper II*, assessment of drug efficacy was done by cure and egg reduction rates at three weeks post-treatment according to the WHO guidelines [46]. In *paper V*, assessment of PZQ efficacy was done by cure rate. The WHO guideline recommends assessing drug efficacy between 14 and 21 days after treatment to avoid parasite-related confounding factors. The chosen follow-up period allows adequate time to release schistosome' eggs following treatment and avoids the effect of immature parasites, which are less sensitive to PZQ [46]. In *paper III*, the assessment of efficacy was done by cure and egg reduction rates at both three weeks and eight weeks post-treatment due to the biology of the parasite development from immature to mature parasite, a process taking 4 to 6 weeks [1, 130]. The eight-week follow-up visit was the main focus of this study and has also been recommended previously [90].

3.3.2 Diagnosis of malaria

A finger prick blood 100 µL was collected and used to detect malaria parasites using SD Bioline malaria Ag P.f/Pan Malaria Rapid Diagnostic Test (MRDT) (SD Standard Diagnostics, Inc, Korea). The results for malaria test were recorded as positive or negative. Studies have

indicated high sensitivity and specificity of the rapid diagnostic test compared to traditional microscopy in diagnosing malaria [131, 132]. Patients who were malaria positive were excluded from the study and treated according to the malaria treatment guidelines [102]. Only those who were malaria negative and intestinal schistosomiasis positive were enrolled in the study. Malaria was also assessed as a predictor of intestinal schistosomiasis infection (*paper I*).

3.3.3 Estimation of haemoglobin concentration

Haemoglobin concentration was determined using the HemoCue Hb 201+ method in g/dL (HemoCue AB Angelholm, Sweden). The method is effective and suitable in resource-limited settings [133]. Haemoglobin concentrations were then classified as mild (11.0 – 11.4 g/dL), moderate (8.0 – 10.9 g/dL) and severe anaemia (< 8 g/dL) based on the WHO guideline [134]. The presence of anaemia was assessed as a predictor of intestinal schistosomiasis infection (*paper I*), cure rate and treatment-associated adverse events (*paper II, III and V*).

3.3.4 Assessment of nutritional status

At the baseline survey, anthropometric measurements such as body weight (kg) and height (cm) were measured from each participant to assess nutritional status (*paper I*). Body Mass Index (BMI) for age Z score (BAZ) and height for age Z score (HAZ) for each participant were then calculated using the WHO Anthro Plus software version 1.0.4 [135]. Children with HAZ and BAZ scores less than two standard deviations were considered stunted and wasted. Stunting and wasting were also assessed as predictors of intestinal schistosomiasis infection and intensity (*paper I*), cure rate and adverse events following treatment (*paper II, III and V*).

3.3.5 Monitoring of adverse events

CRFs were used to collect information regarding adverse events, including their presence and severity. Treatment-associated adverse events were assessed within four hours after drug intake (*paper II and III*). It has been reported previously that most PZQ-related adverse events occur within this time following its intake [136, 137]. Therefore, monitoring of adverse events during this crucial time after the addition of DHP was important. Future studies should consider the assessment of safety for a much longer follow-up time. The treatment-associated adverse events were also linked to pharmacogenetics of PZQ in paper V.

3.3.6 Laboratory analyses of whole blood and plasma samples

3.3.6.1 Quantification of PZQ, enantiomers and trans-4-OH-PZQ concentrations in plasma samples

In paper IV, blood samples (2 mL) were collected at 0, 1, 2, 4, 6 and 8 h post-dose from 64 study participants who received either PZQ and DHP combination (n = 32) or PZQ alone (n = 32). The blood samples were collected into heparinized vacutainer tubes using an indwelling intravenous catheter maintained in the antecubital arm vein and immediately centrifuged at 1,000 rpm for 10 minutes to obtain plasma, which were kept at -80 °C freezer until analyzed. LC-MS/MS equipment (Thermo Scientific) using an enantioselective method was developed

to quantify R-PZQ and S-PZQ concentrations based on a recent validated method described by Kovac *et al*., [138]. Total PZQ concentration was obtained by summing R-PZQ and S-PZQ concentrations. In brief, plasma calibration samples were freshly prepared by spiking blank plasma samples with rac-PZQ and were included in each analytical run. Quality control samples were also prepared by spiking plasma blanks to obtain low, medium, and high concentrations for both R-PZQ and S-PZQ. The quantification range of the method was 1 to 1500 ng/mL for both R-PZQ and S-PZQ.

For extraction of analytes of interest, 100 µL of plasma samples went through protein precipitation with 200µL of internal standard solution (50 ng/mL of rac-PZQ-d11 in methanol) and then vortexed for 10 seconds followed by centrifugation for 5 min at 2100×g. 150 µl of the supernatant was diluted with 75 µL MilliQ water and 5 µL was injected onto the LC-MS/MS system. The chromatographic system was using a Chiralpak AGP 2.0 × 100 mm, 5 µm column (Chiral Technologies Europe, Illkirch, France) with 10 mM ammonium acetate: isopropanol 98:2 (v/v) pH 8 as mobile phase with a flow rate of 0.3 mL/min. The chromatographic run was 22 min, and with the use of the parallel two-channel capacity, injection to injection time was 11 min. R-PZQ eluted first, followed by S-PZQ with a difference of 1.9 min. PZQ was monitored by the transition m/z 313.2>202.9 and the IS rac-PZQ-d11by 324.2>204.1. The calibration curve was constructed by linear regression of the analyte/internal standard area ratios with an applied weighing of $1/x^2$. Accuracy was within ±5% except for LLOQ levels, where it was within ±12%. Precision was below 5 CV% except for LLOQ (below 13 CV %). The final concentrations for R-PZQ, S-PZQ and total PZQ were analyzed by non-compartmental method to obtain the AUCs, $C_{max,}$ half-life (t1/2) and T_{max}. The pharmacokinetic parameters were compared between treatment groups to assess bioequivalence (paper IV).

Due to the long retention time for each sample in the enantioselective method, we also investigated which single sampling time-point better correlate with $AUC_{0\text{-}8h}$ among those treated with PZQ alone for quantification of total PZQ and metabolite *trans*-4-OH-PZQ. We found that plasma samples collected at four hours post-PZQ administration for total PZQ better correlate with $AUC_{0\text{-}8h}$ (Unpublished data).

Therefore, in paper V, plasma samples collected at four hours post-PZQ were analyzed. Only study participants treated with PZQ alone were included in this study (paper II). The UPLC-MS/MS method for quantification of PZQ and *trans*-4-OH-PZQ was adapted from Astra Zeneca laboratories (Sweden) and was recently used by Nleya *et al*., 2019 [139] with minor modifications. In brief, plasma calibration samples were freshly prepared by spiking blank plasma samples with rac- PZQ and *trans*-4-OH-PZQ and were included in each analytical run. Quality control samples were also prepared by spiking plasma blanks to obtain low, medium, and high concentrations for both PZQ and *trans*-4-OH-PZQ. The quantification range of the method was 3.9 to 2500 ng/mL for PZQ and 31.2 to 50000 ng/mL for *trans*-4-OH-PZQ.

For extraction of analytes of interest, 50 µL of plasma samples went through protein precipitation with 150 µL of internal standards solution (25 nM of rac- PZQ -d11 and 25 nM of *trans*-4-OH-PZQ -d5 in 50:50 mixture of acetonitrile: methanol) and the mixture was vortexed for 3 minutes followed by centrifugation for 20 minutes at 3220 g at 4°C. Then, 75 µl of the supernatant was diluted with 75 µL MilliQ water and 5 µL was injected onto the UPLC-MS/MS system for analysis. The chromatographic system was using an Aqcuity

UPLC®HISS T3 column (2.1 × 50 mm, 1.8 µm (Waters, Ireland). The mobile phase consisted of solvent A (0.1% formic acid and 2% acetonitrile in water) and solvent B (0.1% formic acid in acetonitrile) with a flow rate of 0.8 mL/min. The column temperature was maintained at 60° C.

The chromatographic run was 4.7 minutes, starting at 4% of solvent B with an increase to 70% of solvent B at 2.6minutes. From 3.1 minutes the column was washed with 96% of solvent B until 4.1 minutes, with two dips to 4% of solvent B in the middle to ensure efficient washing. Column re-equilibration was done from 4.2 to 4.7minutes, but was in effect longer when including the injection time.T*rans*-4-OH-PZQ eluted first at a retention time of 1.15 minutes followed by PZQ at 1.89 minutes (Figure 6). PZQ was monitored by the transition m/z 313.2>203.1 and the IS rac- PZQ -d11by 324.2>204.1 and for *trans*-4-OH-PZQ by the transition m/z 313.2>203.1 and the IS t*rans*-4-OH-PZQ -d5 by 324.2>204.1. Because of the very high concentrations of *trans*-4-OH-PZQ in the samples, a detuned (sub-optimized) MS method was used by decreasing the collision energy setting for that transition.

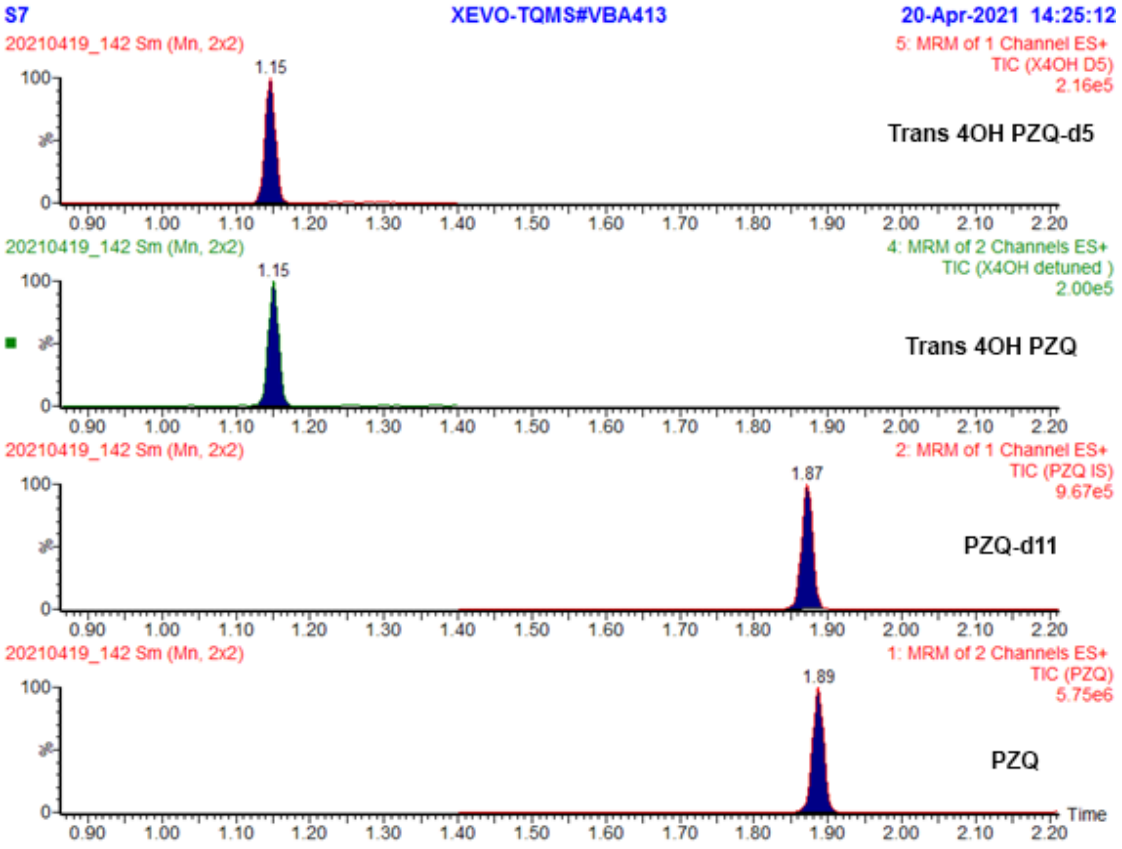

Figure 6. *Chromatography showing retention times and peak shape for PZQ and trans-4-OH-PZQ with internal standards.*

Quantification of PZQ and *trans*-4-OH-PZQ was done using Target Lynx software (Waters). The calibration curves were constructed by linear regression of the analyte/internal standard area ratios, with a quadratic curve fit and an applied weighing of 1/x. A minimum of 12 calibration points were used, and calibrators were injected at start and end of each analysis. Three quality control samples were injected at regular intervals throughout the analyses. The PZQ and *trans*-4-OH-PZQ concentrations were estimated based on the ratio of the analyte peak area to the internal standard area.

Accuracy and recovery of the method was measured from 3 quality control samples each, at low (QCL), mid (QCM), and high (QCH) levels. For PZQ, recovery was 105% for QCL, 87% for QCM, and 100% for QCH at 5, 8.7, and 1 % relative standard deviation (RSD), respectively. For *trans*-4-OH-PZQ the recovery was 104, 109, and 97.11 for the three QC levels, and accuracy was 2.6, 2.7, and 1.9 % RSD. The precision for PZQ was measured by injection of 6 replicates and was 6.7 % RSD at LLOQ, and 4.1 % RSD at QCH. For *trans*-4-OH-PZQ, the area precision was 6.4 % RSD at LLOQ and 5.3 at QCH. The calibration curves for both PZQ and *trans*-4-OH-PZQ had a coefficient of determination (r^2) of >0.98. No carry-over was detected for the compounds analyzed.

Both analytical methods (*paper IV and V*) were partially validated according to the European Medicines Agency Guideline on bioanalytical method validation [140].

3.3.6.2 Collection of the whole blood sample, Genomic DNA isolation and Genotyping

Before drug administration, 2 mL of whole blood samples were collected in an EDTA vacutainer tube from each participant. The blood samples were temporarily stored -80°C at NIMR Mwanza research centre laboratory before being shipped to the Department of Laboratory medicine, Karolinska University Hospital Huddinge (Stockholm) for analysis.

According to the manufacturer's instructions, genomic DNA was isolated from peripheral leucocytes using Qiagen QIAamp DNA Midi Kit (Qiagen GmbH, Germany). The isolated genomic DNA were then temporarily stored at -20°C until time for genotyping.

Genotyping for common variant alleles *CYP3A4 (*1B), CYP3A5 (*3,*6,*7), CYP2C19 (*2,*3,*17) and CYP2C9 (*2,*3)* relevant for PZQ metabolism were done [111]. Genotyping was performed using TaqMan® drug metabolism genotyping assay reagents for allelic discrimination (Applied Biosystems Genotyping Assays) with the following ID numbers for each SNP: C__11711730_20 for *CYP3A4*1B* (−392A>G, rs2740574), C__26201809_30 for *CYP3A5*3* (c.6986A4G, rs776746), C__30203950_10 for *CYP3A5*6* (g.14690G4A,rs10264272), C__32287188_10 for *CYP3A5*7* (g.27131_27132insT rs41303343), C__25986767_70 for *CYP2C19*2* (rs4244285), C__27861809_10 for *CYP2C19*3* (rs4986893), C__469857_10 for *CYP2C19*17* (rs12248560), C__25625805_10 for *CYP2C9*2* (rs1799853), and C__27104892_10 for *CYP2C9*3* (rs1057910). Genotyping was done using 7500 Fast Real-Time PCR (Applied Biosystems, USA).

The pharmacogenetics data were analyzed to obtain the genotype and allele frequencies. The *CYP3A4, CYP3A5, CYP2C19* and *CYP2C9* genotypes were then linked to plasma concentrations (PZQ, *trans*-4-OH-PZQ and metabolic ratio (*trans*-4-OH-PZQ/PZQ)) and schistosomiasis treatment outcomes both efficacy and safety (paper V).

3.4 DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data were collected in paper work and then transferred into a database. The database was created by the Data Management Unit (DMU) of the National Institute for Medical Research, Mwanza Research Centre, using Census and Survey Processing system (CSPro) software (U.S

Census Bureau, USA). Data collection was done by well-trained research assistants of NIMR Mwanza research centre under supervision and guidance of PhD student. The research team was trained on Standard Operating Procedures (SOPs) and Good Clinical and Laboratory Practices (GCLP). The collected data was cleaned and data analysis was done using Statistical Package for Social Sciences (SPSS) Windows version 20 (SPSS, IBM Corp, Armonk, NY, USA) for paper I, II, III and V and R statistical software (version 4.0.2 and PKNCA package version 0.9.4) for paper IV.

Descriptive statistics was used to analyze socio-demographic and baseline characteristics of the study participants (paper I, II, III, IV and V**)**. Categorical variables were presented as proportions, while continuous data were summarized as mean ± standard deviation (SD) or median (Interquartile range – IQR) depending on the normality distribution of data. Chi-square or Fishers exact test was used to assess association between categorical variables (paper I, II, III, IV and V**)**. Independent sample t-test was used to compare the means of normally distributed data, while Wilcoxon Mann, Whitney U test was used to compare continuous data that were not normally distributed (paper I, II, III and IV**)**.

In addition to the above analysis, in paper I, a negative binomial regression model was used to assessing for the predictors of baseline infection intensity (epg) (count outcome variable). This test was chosen over Poisson regression model because there was over dispersion of the epg data (variance > mean). To apply the Poisson regression model, the mean should be equal to the variance. Spearman correlation test was used to assess the relationship between two continuous data i.e. infection intensity (epg) vs haemoglobin concentration. Univariate and multivariate regression analysis was used to assess for the predictors of intestinal schistosomiasis infection (binary outcome variable). All variables with $p < 0.2$ from the univariate analysis were included in the multivariate regression model.

In paper II, univariate and multivariate regression analysis was used to assess for the predictors of cure at three weeks post-treatment. All variables with $p < 0.3$ from the univariate analysis were included in the multivariate regression model.

In paper III, two-way ANOVA was used to compare the baseline infection intensity (epg) between infection intensities (light to heavy infections) and treatment groups. This statistical test is suitable when comparing count variable between two categorical variables. Univariate and multivariate regression analysis was also used to assess for the predictors of cure at eight weeks post-treatment, which was the main focus of this study. Predictor variables with $p < 0.2$ from the univariate analysis were included in the multivariate regression model.

In paper IV, the pharmacokinetics parameter values were log-transformed and then anti-logged to obtain geometric means. The concentrations, C_{max} and AUCs values were dose-normalized by dividing their values by received dose. An independent sample t-test was used to compare the means of log-transformed $AUC_{0\text{-}8h}$. A linear mixed-effect model (stepwise manner) was used to determine subject characteristics influencing Noncompartmental pharmacokinetics parameters values (C_{max} AUCs and half-life). The Geometric mean ratio (GMR) of a

pharmacokinetic parameter was calculated as a ratio of PZQ+DHP (test) to PZQ (control). The 90% CI of the GMR of a pharmacokinetic parameter was used to assess for bioequivalence between treatment arms, where any interval outside 80, 125% was considered not bioequivalent.

In paper V, Pearson's chi-square was used to compare the observed and expected genotypes frequencies according to the Hardy-Weinberg equilibrium. One-way ANOVA was used to compare log-transformed PZQ, *trans*-4-OH-PZQ concentrations and the metabolic ratio between different CYP450 genotypes. Pearson's chi-square or Fisher's exact test depending on test appropriateness was used to assess the association between cure rates and adverse events and CYP450 genotypes. Univariate and multivariate regression analysis was used to assess for the predictors of cure at three weeks and adverse events within four hours post-dose. All variables with $p < 0.2$ from the univariate analysis were included in the multivariate regression model.

In all multivariate regression analyses done in this thesis, a variable with a $p < 0.05$ was considered as a significant predictor. The Hosmer and Lemeshow test was used to assess the goodness of fit of the multivariate models.

3.5 ETHICAL CONSIDERATIONS

Ethical and scientific approval to conduct this study was granted by the Medical Research Coordination Committee (MRCC) of the National Institute for Medical Research, Dar es Salaam Tanzania (Ref. No. NIMR/HQ/R.8a/Vol.IX/2343 and renewals (NIMR/HQ/R.8c/Vol.II/953 & NIMR/HQ/R.8c/Vol.I/1212) and the Muhimbili University of Health and Allied Sciences Institutional Review Board (Ref. No.2016-5-25/AEC/Vol.X/03). The clinical trial was then registered with the Pan African Clinical Trial Registry (PACTR), registration number PACTR201612001914353. We also applied for an ethical permit from the Stockholm Ethics Committee for the work conducted in Sweden (pharmacokinetics and pharmacogenetics studies) (Ref.No.2020-00845).

Before enrolment, comprehensive information about the study was given to all parents/guardians and participating children in the national language (Swahili) and local language (Sukuma). Thereafter, written informed consent from parents/guardians and assent from participating school children were obtained. Participation in the study was purely voluntary, and participants were free to withdraw from the study at any time without any penalty. Participant's IDs were used to make the collected information anonymous to ensure confidentiality.

4 RESULTS AND DISCUSSION

Overall, a total of 830 school-aged children were enrolled in this PhD project. Out of 830 children, 752 (90.6%) tested positive for intestinal schistosomiasis (*paper I*). Of those who tested positive for intestinal schistosomiasis, 84.9% (639/752) were eligible and consented to participate in the clinical trial and followed up to 8 weeks post-treatment, 341 received PZQ alone, and 298 received PZQ and DHP combination therapy (*paper III*). Children who received PZQ alone were also followed at three weeks post-treatment to assess the efficacy and safety of PZQ as per the WHO guideline as presented in Figure 5.

A total of 100 children out of 752 (13.3%) who tested positive for intestinal schistosomiasis declined treatment during the study period, where 57 children declined before and 43 children after random allocation in the test group (Figure 5). The decline rate to treatment of13.3% among those who tested positive for intestinal schistosomiasis in this study may highlight one of the challenges (i.e. low treatment coverage) in the efforts to control and eliminate schistosomiasis in endemic countries [56]. The untreated children will serve as a reservoir of infection and continue transmitting the disease in the community [6]. Unfortunately, this study did not investigate the reasons for the decline of treatment. However, other studies in Africa reported participation in daily economic activities, bitter taste of the drug, school enrollment, and settlement in a village, and previous exposure to drug-related adverse events as significant contributing factors [141, 142]. Therefore, the national NTDs control and elimination programs should not only focus on drug availability and distribution in the at-risk populations but also address the factors contributing to the low treatment coverage.

4.1 Prevalence and correlates of intestinal schistosomiasis infection among school children (paper I)

A total of 830 school-aged children aged 7 – 19 years old were eligible and recruited in the baseline survey. The mean age (±SD) was 11.7 ± 1.9 years. At recruitment, 155 children out of 830 (18.7%) reported pre-treatment abdominal pain. Out of 830 recruited children, 459 (55.3%) had a normally formed stool, while 266 (32%) and 105 (12.7%) had soft and loose stool, respectively.

Prevalence and intensity of intestinal schistosomiasis

Out of the 830 children examined, 752 (90.6%) were infected with intestinal schistosomiasis. Children below the age of 12 years were more infected (64.6%, 536/830) than those above 12 years (26.0%, 216/830) ($p < 0.001$). There was no significant difference in the prevalence of infections between males and females. All children who had either soft (n=266) or loose stool (n=105) at enrolment were found to be infected with intestinal schistosomiasis (Table 1&2 – paper I) ($p = < 0.001$). Out of the 155 who had abdominal pain during enrolment, 140 children (16.9%, 140/830) were infected with intestinal schistosomiasis. However, there was no association between pre-treatment abdominal pain and intestinal schistosomiasis infection

(p=0.89). On both univariate and multivariate logistic regression analysis, lower age was the only significant predictor of intestinal schistosomiasis infection (Table 4 – paper 1).

Overall, the study population was moderately infected with intestinal schistosomiasis, with the median infection intensity of 204 eggs per gram (epg) (IQR 54 – 457) as per the WHO guideline [12]. The majority of the participants had moderate (38.4%) to heavy infections (28.1%), while the light infection was 24.1%. Participants with loose and soft stools were had significantly higher mean epg of stool than those with formed stool ($p < 0.001$) (Figure 1 – paper 1). Based on the negative binomial regression model, being male, having loose stool, and stunting were found as significant predictors of high eggs counts (Table 3 – paper 1).

Prevalence of malaria and association with intestinal schistosomiasis

Out of 830 participants, 824 (99.3%) were available and provided blood samples for malaria testing, where 14 out of 824 (1.7%) were found to be malaria positive. There was no significant difference in malaria prevalence between age groups and sex ($p > 0.05$). A total of 13 out of 14 who tested malaria positive were found to have malaria – intestinal schistosomiasis co-infection, making a co-infection of 1.6% (13/824) in the study population and 92.8% (13/14) among those who were malaria positive. There was no significant association between malaria and intestinal schistosomiasis infections ($p = 0.13$) (Table 2 – paper 1).

Prevalence of anaemia and association with intestinal schistosomiasis

Like in malaria, 824 out of 830 (99.3%) of the children were available and provided blood samples to estimate haemoglobin concentration in g/dL. Out of 824, 203 (24.6%) of the children were found to be anaemic (haemoglobin concentration < 11.5 g/dL). Severe anaemia was observed in 2.3% of the children (19/824). The median haemoglobin concentration of the study population was 12.6 g/dL (IQR 11.5 – 13.5). Children not infected with intestinal schistosomiasis had slightly higher haemoglobin concentration (12.8 g/dL) than infected ones (12.5 g/dL) ($p = 0.93$) (Figure 2 – paper 1). There was no association between anaemia and intestinal schistosomiasis infection ($p = 0.36$).

Prevalence of undernutrition and association with intestinal schistosomiasis

Prevalence of stunting and wasting were assessed in this study. A total of 241 out of 830 (29.0%) and 94 out of 830 (11.3%) were found to be stunted and wasted, respectively. There was no significant association between intestinal schistosomiasis infection and neither stunting ($p = 0.53$) nor wasting (0.41). However, stunting was found to be a significant predictor of high egg counts among those infected (Table 3 – paper 1).

The high prevalence and intensity of intestinal schistosomiasis observed in the study area despite five MDA rounds were similar to previous studies conducted around the Lake Victoria basin [27, 28]. Similar results were reported from other endemic areas in Sub-Saharan Africa [143]. These findings indicate that there are either hotspots or continuous transmission of the disease in the communities despite ongoing interventions. Lack of vaccine to complement

preventive chemotherapy has been sought as one of the major factors for the continued disease transmission in endemic countries [144]. Other factors include poor sustainability of MDA and vector control programs, especially in an area with high transmission and fragmented treatment coverage [56]. E.g. preschool children are excluded from the MDA campaign can serve as a reservoir of infection [145], low knowledge on disease transmission and prevention, poor sanitation and hygiene and lack of safe and clean water [146] as the communities rely on Lake's water for all human needs. There was no MDA in 2016, a year before this study has commenced in the study area. The interruption of MDA (single-dose PZQ), which in itself seems not sufficient enough to control the disease in such settings with high transmission, may have contributed to the observed high prevalence of the disease.

Pre-adolescents (≤ 12 years) were also found to be more infected than adolescents (> 12 years), which might indicate a carry-over of infection as preschool children are not involved in MDA. Even if a child is infected at three years old has to wait for at least 3 to 4 years to start primary education and get treatment [145]. Recent studies have shown a correlation between schistosomiasis infection prevalence between school-aged children and pre-school children [147]. Therefore, when the prevalence of infection is high in school children, it is equally the same in pre-school children. Early integration of pre-school children in mass drug administration presents a key step towards disease control and elimination efforts [148, 149].

Apart from preventive chemotherapy, previous studies have also indicated that control and elimination of schistosomiasis in endemic countries can be achieved by improving sanitation and hygiene and access to clean and safe water [150-152]. These factors are far behind in Sub-Saharan Africa; therefore, eliminating schistosomiasis as a public health problem by 2030 may be a premature target in the region.

The inability of PZQ to act against immature worms may be another major factor for the continued transmission of the disease in endemic countries [48, 72, 153], where the individuals are constantly infected and harbour more immature parasites. These juvenile parasites are left behind during PZQ MDA, and after 4 – 6 weeks, they attain their reproductive maturity and start laying eggs [1]. Therefore, there is a pressing need for an alternative treatment strategy that can either act on juvenile parasites or have a different mechanism of action to complement PZQ. So far, no documented report of PZQ resistance; however, an alarming threat of PZQ tolerance/resistance has been reported from field studies from SSA [70]. Hence the need to continuously assess disease prevalence and PZQ efficacy in the endemic setting as recommended by the WHO [46]. The WHO recommends assessing drug efficacy if the prevalence and intensity of infection are still high despite preventive chemotherapy [46].

Intestinal schistosomiasis infection was not significant associated with malaria, anaemia and undernutrition in the present study. Similar results have been reported previously [27, 28] except for anaemia which is associated with *Schistosoma haematobium* infection [26]. Nevertheless, the burden of anaemia and undernutrition continued to be high among school children, similar to what was reported in previous studies [27, 28]. These conditions may limit children from getting a better education.

4.2 Efficacy and safety of praziquantel for treatment of *Schistosoma mansoni* infection among school children (paper II)

A total of 341 *Schistosoma mansoni* infected children (7-17 years) and treated with single-dose PZQ (40 mg/kg body weight) were enrolled in this study and followed for three weeks as per the WHO guidelines [46]. The mean age (±SD) of the population was 11.8 ± 1.7 years. About 20.8% (71/341) of the participants had reported abdominal pain before treatment. The majority of the participants had moderate infection intensity (44.6%), as summarized in Table 1 of paper II. The median infection intensity of the study population was 222 epg (IQR 96 – 471).

Efficacy of PZQ by cure and egg reduction rates

The efficacy of PZQ was assessed based on cure rate and egg reduction rate (ERR) at three weeks post-treatment as per the WHO guidelines [46]. The cure rate was defined as the proportion of treated patients who were eggs positive at baseline and turned eggs negative at three weeks of the post-treatment follow-up visit. The cure rate was 81.2% (277/341) (95% CI 76.8, 85.3%). There was no significant association between cure rate and age, sex, baseline infection intensity, stunting, wasting and anaemia (Table 2&4 – paper II). There was a significant drop in the level of infection intensity before and after treatment from light to heavy infection (Table 3 – paper II), e.g. heavy infection has dropped from 29.9% to 0.9% in the study population. The egg reduction rate was found to be 95% (95% CI 92.7 – 97.3%). There was no significant association between egg reduction rate and age ($p = 0.12$) and sex ($p = 0.45$).

The observed cure and egg reduction rates in this study were within the acceptable range. Cure rate between 60% and 90% for *Schistosoma mansoni* [93] and an ERR of > 90% are considered satisfactory according to the WHO guideline [46]. Based on ERR, these results indicate that PZQ is still efficacious against *Schistosoma mansoni*. We have based on the ERR findings for evaluation of PZQ efficacy because the cure rate has been reported not to be a good indicator of PZQ efficacy, as detailed by Montresor A. *et al.* [154]. The use of ERR to assess PZQ efficacy has also been recommended by the WHO [46]. However, the lack of cure in 18.8% of the study participants points to the need for further research to optimize PZQ efficacy. Some studies have indicated that increased PZQ MDA rounds may be associated with low parasitological cure, especially with *Schistosoma mansoni* [91]; hence close monitoring of the drug efficacy is vital for early detection of reduced efficacy or drug resistance. This is especially important because, to date, there is no alternative drug to PZQ for treatment and control of schistosomiasis.

PZQ treatment-associated adverse events (safety)

Treatment-associated adverse events were assessed within four hours of PZQ administration, as reported previously in other studies [75, 137]. This is because, during this timeline, the majority of adverse events are observed following PZQ intake. Overall, a total of 28.7% (97/341) of the participants experienced adverse events. Post-treatment abdominal pain was experienced and reported by 26.7% (91/341) of the treated children, while 1.8% (6/341) reported vomiting. However, almost all adverse events were mild and transient. No serious

adverse was observed in this study, particularly neurological symptoms including dizziness, drowsiness or fainting. Post-treatment abdominal pain was significantly associated with baseline infection intensity, whereby children who had moderate (25.7%) to heavy infection (40.2%) experienced more adverse events compared to those with light infection (12.6%). Vomiting was significantly associated with anaemia, whereby anaemic children had more vomiting than non-anaemic children (Table 6 – paper II).

PZQ has been associated with adverse events following intake. However, as observed in this study and reported in most previous studies [86, 155], most of PZQ associated adverse events were mild and resolve within four hours of drug administration. In this study, we have also observed a few adverse events. This might be due to the pre-treatment meal given to the children before drug administration as recommended by the WHO [46]. Therefore, based on the findings, PZQ is still safe and well-tolerable to the treated children. However, clinical examination may be considered before treatment to identify anaemic children who may need special monitoring following PZQ intake since vomiting was significantly associated with anaemia. Unfortunately, the Lake Zone has been reported to have a high prevalence of schistosomiasis, and therefore, the need for PZQ MDA has been reported to have a high prevalence of anaemia as well [27, 28]. Similar to previous studies, post-treatment abdominal pain was associated with pre-treatment infection intensity [137]. Heavily infected children experience adverse events more than those with light to moderate infection.

For that reason, assessment of adverse events during PZQ efficacy studies remains important since most studies estimated sample size based on the efficacy section, and it may be difficult to observe rare adverse events. There is a lack of studies based on active cohort event monitoring to assess the safety of medicines used to control and eliminate neglected tropical diseases in Sub-Saharan Africa, contrary to what is recommended by the WHO [82]. For example, recently, visual impairment was linked with PZQ MDA in the Eritrean population [156]. The WHO is making a close follow-up on the matter with proper methodology (i.e. active cohort monitoring) to establish a causal association [157]. Furthermore, studies to investigate the effect of pharmacogenetics variations on safety following PZQ are lacking despite the varying degree of reported adverse events between treated populations [85, 123]. In this thesis, this knowledge gap has been evaluated and is presented in paper V.

4.3 Efficacy and safety of praziquantel and dihydroartemisinin-piperaquine combination versus praziquantel alone for treatment of intestinal schistosomiasis: non-inferiority trial (paper III)

Out of 752 children who tested positive for intestinal schistosomiasis, 682 (90.7%) were eligible and consented to participate in the trial. These were randomized (1:1) based on infection intensity, where 341 were randomly allocated to receive PZQ alone, and 341 received PZQ and DHP combination. Since participation in the study was voluntary, and participants were free to withdraw from the study at any time, 43 children declined treatment from the PZQ and DHP combination arm after allocation (Figure 5). Therefore, 639 children were involved in the trial, of whom 341 children were treated with PZQ alone and 298 children with PZQ and

DHP combination. At enrollment, most participants had moderate infection intensity (43.8%) (Table 1 – paper III). After randomization, there was no significant difference between treatment groups ($p > 0.05$) except for stunting, where those on the PZQ arm were more stunted (34.3%) than PZQ and DHP arm (21.8%) ($p < 0.001$). However, there was no significant association between cure rates and stunting ($p > 0.05$).

Cure and egg reduction rates between treatment groups

At 3 weeks post-treatment, cure rates were 88.3% (95% CI 84.1- 91.4%) and 81.2% (95%CI 76.7 – 85.0%) in the PZQ and DHP combination and PZQ, respectively ($p = 0.01$). The difference in cure rate between treatment groups was 7.1% (95%CI of the difference 1.48 – 12.6%). At 8 weeks post-treatment, cure rates were 81.9% (95%CI 77.1 – 85.8%) and 63.9% (95CI 58.7 – 68.8%) in the PZQ and DHP combination and PZQ, respectively ($p < 0.001$). The difference in cure rate between treatment groups was 18% % (95%CI of the difference 11.2 – 24.6%). Therefore, the overall cure rates at both follow-up visits were significantly higher in the PZQ and DHP combination than in the PZQ arm. At eight weeks post-treatment, cure rates were significantly higher in the PZQ and DHP combination than in PZQ across all levels of infection intensity (light to heavy infection) ($p < 0.05$). For instance, among those with heavy infections, at eight weeks post-treatment, cure rates were 75.9% in the PZQ and DHP combination versus 59.8% in the PZQ arm ($p = 0.02$) (Table 3 – paper 3). On both univariate and multivariate regression analysis to identify predictors of cure at eight weeks post-treatment, only type of treatment received was found to be a significant predictor ($p < 0.001$) (Table 4 – paper 3). At 8 weeks of post-treatment, ERR were also significantly higher in PZQ and DHP combination (93.6%, 95%CI 90.8 – 96.4%) than in PZQ arm (87.9%, 95%CI 84.4 – 91.4%) ($p = 0.01$). At three weeks of post-treatment, ERR were not significantly different between treatment groups (Table 5 – paper 3).

These findings indicate that the combination of PZQ and DHP is more efficacious than PZQ alone to treat schistosomiasis. Similar results have been reported when PZQ and artemether or artesunate were co-administrated to treat schistosomiasis [97-99]. The combination therapy of PZQ and DHP can be considered for treatment and control of schistosomiasis, especially in the areas with high transmission of the disease and in malaria-endemic settings. As the risk of artemisinin resistance against malaria parasite is high with the use of artemisinin as monotherapy combined with PZQ [56], in settings where both schistosomiasis and malaria are endemic, the use of ACTs such as DHP in combination with PZQ may be a better option. The two diseases remain a major public health challenge, particularly in Sub-Saharan Africa [158]. Studies to investigate the usefulness of mass treatment of malaria in school children have been carried out in some Sub-Saharan countries. Intermittent preventive treatment (IPT) with mass administration of sulphadoxine-pyrimethamine plus piperaquine or amodiaquine in Congo [159], in Kenya [160], and Mali [161] and mass treatment using DHP in Uganda [162] in school children has shown promising results in terms of malaria prevention. The interventions also indirectly improved the burden of anaemia among school children.

The fact that the two diseases are overlapping in most countries in Sub-Saharan Africa, and children remain the most vulnerable population [163], studies have also been conducted to integrate mass treatment of malaria in existing NTDs control programs. A pilot study in Malawi has investigated the co-administration of PZQ and albendazole for NTDs and artemether Lumefantrine (ALU) for malaria and reported that the co-administration was well tolerated by the school children [164]. In Ghana, apart from reducing malaria prevalence and improving anaemia, the co-administration of malaria and helminths treatments was also associated with increased school participation and performance [165]. However, due to the complex administration schedule of ALU, DHP, which is once a day for three days, can be a good option in combination with PZQ for schistosomiasis and malaria control in school children [102]. DHP has a long half-life which offers much longer protection against malaria [107] and has not been extensively used for clinical management of malaria in Sub-Saharan Africa and hence less drug pressure [106]. Therefore, the national schistosomiasis and malaria control programs in Sub-Saharan Africa should consider working in collaboration and use PZQ and DHP combination to halt the burden of the two diseases, particularly in school children.

On the other hand, repeated PZQ doses spaced 3-6 weeks apart have also been investigated for the treatment and control of schistosomiasis and shown better treatment outcomes by killing immature parasites left behind during the first dose of PZQ administration [71]. Although there is still ongoing debate regarding the usefulness of repeated PZQ doses versus cost and risk of accelerating PZQ resistance, its use in high transmission areas of the disease where a single annual PZQ dose is obviously not adequate cannot be underestimated. However, it is an obvious fact that repeated PZQ doses will be costly in endemic countries with limited resources where even annual single-dose PZQ administration is not sustainable. Furthermore, repeated doses will definitely increase PZQ use, and it has been reported that increased use of PZQ in MDA is associated with low cure rates and may accelerate the risk of parasites developing resistance against PZQ [91]. Indeed, PZQ resistance is a major concern among experts in the field since there is no alternative drug [92, 166]. Since the emergence of drug-resistant mutants is a direct result of selective pressure by repeated drug use, the chance of resistance to two drugs with a different mechanism of action is lower than single drug use, provided that resistance mechanisms are independent [94, 95].

Accordingly, combination drug therapy is the current standard treatment strategy for infectious diseases such as tuberculosis, HIV and malaria, and it is a way to go for NTDs. The risk of parasite resistance associated with repeated PZQ dose as a strategy can be overcomed with the proposed combination therapy of PZQ and ACTs such as DHP. PZQ is speculated to act mainly by disrupting the Ca^{2+} channels and finally the parasite' outer tegument, while artemisinin works by inhibiting the schistosome-specific thioredoxin glutathione enzyme and/or reduction in schistosome glycogen and protein content [72, 167]. Thioredoxin glutathione enzyme has been reported to be a major potential target of drugs for NTDs [168]. However, despite better treatment outcomes shown by PZQ and DHP, further studies to investigate cost-effectiveness, feasibility in a wide community implementation and the usefulness of this combination therapy are important as DHP remains one of the key antimalarial drugs worldwide.

Treatment-associated adverse events

Overall, 197 out of 639 treated children (30.8%, 95%CI 27.2 – 34.4%) experienced adverse events. Abdominal pain was observed in 27.1% (173/639) while vomiting in 3.4% (22/639) of the children. In the PZQ arm, a total of 97 out of 341 (28.4%, 95%CI 23.7 – 33.3%) of the children experienced adverse events. A slightly higher percent (33.6%, 95%CI 28.2 – 38.9%) (100/298) was observed in the PZQ and DHP arm. Without considering a kind of adverse event experienced by treated children, overall, there was no statistically significant difference in the incidence of adverse events between treatment groups ($p = 0.16$). However, stratified by type of adverse events, vomiting was significantly higher among those treated with PZQ and DHP combination (5.4%) than PZQ alone (1.8%) ($p = 0.01$) (Table 6 – paper 3). Conversely, almost all adverse events observed were mild and transient, and no serious adverse event was experienced by treated children in both treatment groups.

The findings suggest that the overall PZQ and DHP combination is equally safe to PZQ alone. Similar results were reported when PZQ was combined with artesunate as a monotherapy [97]. In this study, overall safety has been further maintained even with the addition of DHP. This finding is significant because both efficacy and safety need to be maintained. PZQ administration has been associated with adverse events, including vomiting (ref). DHP has also been associated with nausea and vomiting in about 1.6 – 6.9% of the treated patients [169]. In Rwanda, DHP administered among younger children (12-59 months) was also safe and tolerable [170]. Similar findings have been reported among Tanzanian children [171]. Therefore, increased vomiting observed may be due to the additional effect of DHP. However, adverse events following DHP alone administration were reported to be mild and transient, and no serious adverse events were reported [169]. Similar findings have been observed in this study, even in the presence of PZQ. This study has demonstrated an early safety profile of the PZQ and DHP combination (within four hours of drug intake). We recommend future studies to assess safety for a much longer follow-up time. In a wide community MDA using PZQ and DHP combination therapy, an active cohort event monitoring should the deployed for safety monitoring.

4.4 Effect of dihydroartemisinin-piperaquine on the pharmacokinetics of praziquantel (paper IV)

A total of 64 *Schistosoma mansoni* infected children were included in this study, where 32 participants were recruited in each treatment arm. Study participants were randomly selected from those who participated in the clinical trial (paper III). The overall mean age (±SD) of the studied population was 12.7±1.8 years. The overall median infection intensity (range) was 225 (56-552). The infection intensity distribution was as follows; light infection 23 participants (35.8%), moderate infection 19 participants (29.7) and heavy infection 22 participants (34.4%). There was no significant difference for the baseline characteristics between the two treatment groups ($p > 0.05$) (Table 1 - paper IV).

This study has found a significant drug-drug interaction between PZQ and DHP following their co-administration to treat schistosomiasis. The 90% CI of the geometric mean ratios of AUCs and C_{max} of PZQ+DHP/PZQ for total PZQ, R-PZQ and S-PZQ were outside an acceptable bioequivalent interval of 0.80, 1.25. The geometric mean of AUCs and C_{max} of total PZQ, R-PZQ and S-PZQ were higher among children treated with PZQ and DHP combination than those treated with PZQ alone (Table 2, 3 & 4 – paper IV). These findings indicate that the systemic drug exposure of both PZQ and its enantiomers was increased by co-administration of DHP.

Our findings is supported by a report by Lanusse C *et al*., 2018 on the strategies to optimize the efficacy of antihelminthic drugs, where pharmacokinetics optimization to enhance parasite exposure was reported [172]. PZQ and DHP combination produce drug-drug interactions which result in a synergistic effect and hence greater therapeutic efficacy than PZQ alone [78]. The bioavailability of a drug can be affected by drug-drug interactions through competitions for protein binding sites. Therefore, CYPs are a potent site of drug-drug interactions between drugs which share the same metabolic pathways or co-administration of CYPs inhibitors or inducers [78]. Induction or inhibition of the CYP450 enzymes can also result in drug-drug interactions of clinical significance, resulting in treatment success/failure or increased risk of adverse events [173]. Dihydroartemisinin is eliminated mainly by phase 2 metabolism, i.e. glucuronidation. Therefore, the observed drug-drug interaction between PZQ and DHP is most likely due to PZQ metabolism inhibition by piperaquine. Both PZQ and piperaquine undergo phase 1 metabolism via CYP450, primarily CYP3A4. Chloroquine, a 4- aminoquinoline similar to piperaquine [174], has been reported to inhibit PZQ metabolism [114]. Therefore, inhibition of PZQ metabolism by piperaquine resulted in increased AUCs and C_{max} of PZQ. Higher total AUC of PZQ has been linked n increased parasitological cure [85]. The increased systemic PZQ exposure has also been reported when PZQ was administered with CYP450 inhibitor i.e. ketoconazole [139]. However, DHP has more advantages than ketoconazole, including antischistosomal activity against juvenile worms and protection against malaria.

Our results further support the reported high cure rates in a recent clinical trial where superior therapeutic efficacy was observed among children treated with PZQ and DHP combination [175]. The increased PZQ bioavailability in the PZQ and DHP combination therapy serves as an additional mechanism for enhancing the performance of PZQ apart from the killing of both mature and immature worms. Figure 7 summarizes factors affecting the activity of an antihelminthic drug. Therefore, the recommended PZQ and DHP combination therapy is supported by both observed better treatment outcomes and pharmacokinetic data.

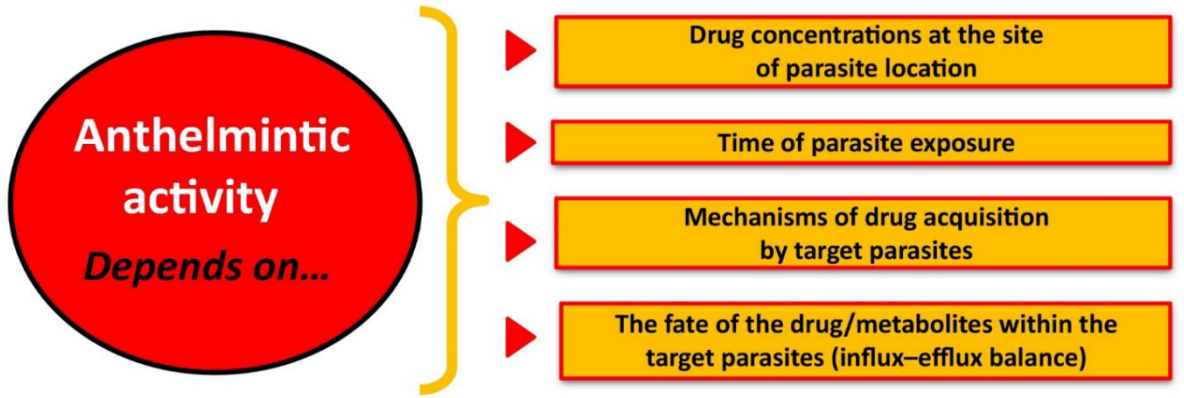

Figure 7. *Parasite and drug factors affecting the activity of the antihelminthic drugs. Source: Lanusse C et al., 2018 [172].*

The findings from this pharmacokinetic study have opened room for further research in the field. The fact that the combination therapy increases PZQ systemic exposure, there is a chance to investigate the effectiveness of a low PZQ dose (< 40mg/kg) combined with DHP to treat schistosomiasis. This approach will reduce not only PZQ pills burden but also bitter taste. On the other hand, the prevalence of schistosomiasis in preschool children has been shown to correlate with that of school children in endemic countries [147]. In response to that, the WHO recommended PZQ treatment among preschool children after several studies reported that the drug is safe in this age group [149, 176]. However, pharmacokinetic studies have shown that AUCs of PZQ and its enantiomers in *Schistosoma mansoni* infected preschool children are higher than school-aged children [138]. In general, preschool children metabolize PZQ slowly [78]. Therefore, further studies are needed in preschool children to assess PZQ exposure and tolerability in PZQ and DHP combination therapy.

4.5 Effect of pharmacogenetics variations on praziquantel plasma concentration and schistosomiasis treatment outcomes (paper V)

A total of 340 study participants were enrolled in this study. Sub-study 5 was aimed to assess the impact of pharmacogenetics variation of PZQ on plasma drug concentrations and intestinal schistosomiasis treatment outcomes (efficacy and safety). The median age (range) of the study participants was 12 years (7-17). The median infection intensity (IQR) of the study population was 222 epg (96-468) (Table 1 – paper V). In this study, a pre-treatment whole blood sample (2 mL) was collected from 340 study participants for DNA isolation and genotyping. Genotyping for *CYP3A4*1B, CYP3A5 (*3,*6,*7), CYP2C19 (*2,*3,*17)* and *CYP2C9 (*2,*3)* alleles that are relevant for PZQ metabolism was done using Real-time PCR [111]. Plasma samples were collected at four hours post-PZQ administration from 287 study participants for quantification of PZQ and metabolite – *trans*-4-OH-PZQ concentrations. To the best of my knowledge, this is the first study to assess the effect of pharmacogenetics variations of PZQ on plasma drug concentrations and schistosomiasis treatment outcomes.

Our findings indicate that there was a significant association between *CYP2C19* genotype and plasma PZQ concentration, and its metabolic ratio (*trans*-4-OH-PZQ/PZQ). PZQ concentration was significantly higher among children carrying *CYP2C19 (*2, *3)* alleles than the wild type (*CYP2C19 *1/*1*) and *CYP2C19 *17* carriers (ultra-rapid metabolizers) ($p = 0.04$). The metabolic ratio was significantly higher among *CYP2C19 *17* carriers than *CYP2C19 (*2, *3)* carriers ($p = 0.01$) (Table 3 – paper V). These findings indicate that CYP3A4/5 are not the major metabolic pathways for the formation of 4-OH-PZQ metabolite in humans. In fact, in vitro studies have reported CYP2C19 as a major metabolic pathway for the formation of 4-OH-PZQ metabolite [177]. Similar findings have been reported recently where CYP3A4/5 were reported to be involved in the formation of X-OH-PZQ metabolite and not 4-OH-PZQ [139].

Our study found no significant impact of *CYP3A4*, *CYP3A5*, *CYP2C19,* and *CYP2C9* genotypes on schistosomiasis treatment efficacy at three weeks post-treatment ($p > 0.05$). Despite CYP3A4 being the major metabolizing enzyme for most drugs used to treat tropical infections, including PZQ, *CYP3A4* genotype was not associated with schistosomiasis treatment efficacy (Table 4 – paper V). Although not statistically significant, those carrying *CYP3A4* defective alleles i. e. *CYP3A4*1B* were more cured than those with wild type genotype (*CYP3A4*1/*1*) (Table 4 and 5). This finding suggests a low CYP3A4 enzyme activity in those carrying *CYP3A4* defective alleles. Similarly, children carrying *CYP3A5* defective alleles *(*3, *6, *7)* were more cured than the wild type (*CYP3A5*1/*1)* ($p > 0.05$) (Table 4 - paper V). *CYP3A5* defective alleles *(*3, *6, *7)* are associated with reduced CYP3A5 enzyme activity [115, 178]. Although *CYP2C19* genotype significantly affect PZQ concentration and its metabolic ratio, *CYP2C19* genotype was not significantly associated with schistosomiasis treatment efficacy.

In this study, we observed no significant effect of *CYP3A4*, *CYP2C19* and *CYP2C9* genotypes on treatment-associated adverse events ($p > 0.05$). However, a border line association was observed between *CYP3A5* and adverse events ($p = 0.048$), where children carrying *CYP3A5* defective alleles *(*3, *6, *7)* had higher incidence of adverse events than the wild type (*CYP3A5*1/*1).* On multivariate logistic regression model, baseline infection intensity was found to be the only significant predictor of adverse events following PZQ treatment. Heavily infected children had significantly more incidence of adverse events compared to those with mild and moderate infections ($p < 0.05$) (Table 6 – paper V). Our findings were contrary to reports from other infectious diseases such as HIV and Tuberculosis, where genetic variations in CYP450 were associated with adverse events [120, 121]. More pharmacogenetics studies of PZQ are needed to generate additional evidence in this field.

5 CONCLUSIONS AND RECOMMENDATIONS

Our findings contribute to the global strategies and efforts in the fight against neglected tropical diseases such as schistosomiasis. The most important conclusions and recommendations of the PhD research project in improving the treatment of schistosomiasis include the following:

- The burden of intestinal schistosomiasis remains high despite ongoing interventions in the study area. We recommend regular assessment of the prevalence and infection intensity in affected areas for targeted implementation of preventive chemotherapy and other intervention measures by the National NTDs Control Program. The surveillance of schistosomiasis prevalence should not only focus on school children but also involve other key populations such as pre-school children.

- Single-dose praziquantel is safe and efficacious against *Schistosoma mansoni* infection. However, lack of cure in about 18% of the treated children points to the need for treatment optimization research. We also recommend regular monitoring of the efficacy of praziquantel for early detection of reduced efficacy or drug resistance would be critical to ensure control and prevention of schistosomiasis in high burden settings.

- Praziquantel and dihydroartemisinin-piperaquine combination therapy have superior efficacy than praziquantel alone for the treatment of schistosomiasis. The combination therapy is equally safe to praziquantel alone when used to treat schistosomiasis among school children. We recommend the adoption of this combination therapy for the treatment and control of schistosomiasis in endemic countries. Since schistosomiasis control and elimination is multifactorial, the use of the praziquantel and dihydroartemisinin-piperaquine combination therapy should be coupled to other control strategies, including supply of safe and clean water and improvement in sanitation and hygiene. Future studies to assess the acceptability, feasibility and cost-effectiveness of this combination therapy is recommended.

- Dihydroartemisinin-piperaquine increases the systemic exposure of praziquantel and hence its therapeutic efficacy. The increased systemic praziquantel exposure is an additional mechanism by which the therapeutic efficacy of the combination therapy increased, apart from killing both immature and mature stages of the parasite. Since the same combination therapy could be similarly beneficial to pre-school children, we recommend studies to assess the tolerability of combination therapy in this age group. This is because in previous studies, pre-school has been shown to have higher AUC compared to school children due to slow metabolism.

- *CYP2C19* genotype significantly affects praziquantel concentration and metabolic ratio (*trans*-4-OH-praziquantel/praziquantel) following praziquantel treatment. No significant effect of *CYP3A4*, *CYP2C19*, and *CYP2C9* genotypes on schistosomiasis treatment efficacy and adverse events was observed. However, a border line association between *CYP3A5* genotype and adverse events was observed. Future pharmacogenetics studies should consider evaluating the impact of *CYP1A2* and *CYP2D6* genotypes on plasma drug concentrations and schistosomiasis treatment outcomes.

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